

UNIVERSITÉ DU QUÉBEC À MONTRÉAL

THE EFFECTS OF BASAL RESOURCE MANIPULATION
ON THE ABUNDANCE AND NUTRITIONAL STATE
OF FRESHWATER CRUSTACEAN ZOOPLANKTON

MÉMOIRE

PRÉSENTÉ

COMME EXIGENCE PARTIELLE

DE LA MAÎTRISE EN BIOLOGIE

PAR

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UNIVERSITÉ DU QUÉBEC À MONTRÉAL

LES EFFETS DE LA MANIPULATION DES RESSOURCES DE BASE SUR
L'ABONDANCE ET L'ÉTAT NUTRITIONNEL DE ZOOPLANCTON
CRUSTACÉ

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RÉSUMÉ

Les lacs d'eau douce subiront une augmentation de matière organique terrestre associée aux changements climatiques dont les effets sur le réseau trophique aquatique sont inconnus. Les profils d'acides gras (AG) peuvent fournir une approche mécanistique pour la compréhension des réponses de la communauté de zooplancton face aux changements environnementaux comme une augmentation de carbone organique dissous (humique) et une augmentation d'éléments nutritifs. Avec l'utilisation de mésocosmes dans un lac des Laurentides (QC), des voies divergentes d'acquisition des ressources par le zooplancton ont été créées favorisant ou défavorisant la consommation d'algues. Une seule communauté de zooplancton a été exposée aux traitements: control, nutriments (azote + phosphore), humique et humique + nutriments. La durée de l'expérience était de 6 semaines, et les variables réponses étaient l'abondance, le biomasse, la diversité et la composition des espèces ainsi que leur profil lipidique des principaux taxons zooplanctoniques. Comparé au traitement ambiant, l'environnement favorisant le phytoplancton (traitement nutriments) a induit une augmentation de l'abondance relative du zooplancton, de la diversité, ainsi que des AG liés à la fécondité. Les traitements humique et humique + nutriments ont diminué l'abondance, le biomasse et la diversité des espèces et engendré une perte des AG liés au succès de reproduction et fécondité. La perte des AG essentiels associés à l'augmentation de carbone dans les lacs peuvent expliquer les changements écologiques dans les communautés de zooplancton crustacé, et peuvent avoir des conséquences nutritionnelles négatives pour les consommateurs d'ordre supérieur dans la chaîne alimentaire aquatique.

Mots Clés: acides gras, changements climatique, écosystèmes d'eau douce, limnologie, zooplancton

ABSTRACT

Freshwater lakes may suffer an increase in terrestrial organic matter associated with climate change, of which the effects on the aquatic food web are unknown. Fatty acid (FA) profiles can provide a mechanistic approach to understanding the responses of the zooplankton community in response to environmental changes such as an increase in dissolved organic carbon (humic) and an increase of nutrients. With the use of in-lake mesocosms in the Laurentians (QC), different resource acquisition pathways for zooplankton were created by encouraging or discouraging the consumption of algae. A single zooplankton community was exposed to treatments: control, nutrients (nitrogen + phosphorus), humic and humic + nutrients. The duration of the experiment was 6 weeks and the response variables were abundance, biomass, diversity and species composition, and FA profiles of major zooplankton taxa. Compared to the ambient treatment, environments favoring phytoplankton (nutrient treatment) induced an increase in the relative abundance of zooplankton, species diversity, and FA linked to fertility. Humic and humic + nutrients treatments decreased abundance, biomass and species diversity and resulted in a loss of FA related to reproductive success and fertility. The loss of essential FA associated with the increase of carbon in lakes may explain the ecological changes in the crustacean zooplankton communities, and may have negative nutritional consequences for higher order consumers in the aquatic food chain.

Key Words: Fatty acids, climate change, freshwater ecosystems, limnology, zooplankton

CHAPITRE I

1.1 Introduction to research

Freshwater ecosystems in northern regions may experience increases in terrestrial-source nutrients and dissolved organic carbon (DOC) that could be augmented by both land use change and increased precipitation associated with climate change (Jeppesen et al. 2009). Dissolved organic carbon can be produced naturally within a freshwater lake (autochthonous) or can be loaded into lakes via terrestrial input (allochthonous) (Larsen *et al.*, 2009). These effects are anticipated to have cascading effects on the quality of basal resources for primary consumers, with potential consequences for the abundance and nutritional state of crustacean zooplankton. Crustacean zooplankton are a key energy link in aquatic food webs as they occupy an intermediate trophic position between basal resources (phytoplankton and heterotrophically-upgraded bacterial resources) and vertebrate consumers (Thorp and Covich 2010). My thesis tested how enriched and suppressed basal resource pathways resulting from altered nutrient and allochthonous DOC inputs can change crustacean zooplankton communities, both from an ecological and biochemical perspective.

Anthropogenic-source terrestrial runoff has been the subject of interest of many limnological studies, as they can have profound effects on food webs in freshwater aquatic systems (Edmonson & Lehnman, 1979; Schindler *et al.*, 1977;

Tranvik *et al.*, 2009; Karlsson *et al.* 2009; Lennon *et al.*, 2013). Elevated concentrations of nutrients and DOC are characteristic of agricultural and urban development as fertilizers and storm water leech into rivers and lakes (Paul & Meyer, 2001; Jeppesen *et al.*, 2009; Edmonson & Lehman, 1981). Eutrophication of freshwaters can result from increased nutrient (phosphorus and nitrogen) deposition via fertilizers (Schindler *et al.*, 1977; Edmonson & Lehnman, 1979). Freshwater systems are phosphorus limited, and, to some extent nitrogen limited, and when external input of nutrients occurs, this limitation is removed (Edmonson & Lehnman, 1979). With eutrophication, algal blooms occur, changing the quantity and quality of basal resources available to aquatic biota, and changing the physico-chemical environment, which in turn can have cascading effects throughout the food web (Paul & Meyer, 2001; Jeppesen *et al.*, 2009; Edmonson & Lehman, 1981).

In combination with land use, there is evidence that climate change could increase terrestrial sources of nutrient and DOC to freshwater boreal ecosystems. Dissolved organic carbon (DOC) in aquatic systems derives from two distinct sources: autochthonous primary production within the system or allochthonous (terrestrial) organic carbon entering the system from watershed sources (Cole *et al.*, 2002). Increases in allochthonous DOC in lakes are predicted to occur via increases in precipitation and terrestrial runoff, changes in the duration of winter ice cover, altered thermal stratification, warmer surface waters, and changes in water chemistry (Larsen *et al.*, 2011). Larsen *et al.* 2011, applied mathematical models to long term data sets of major parameters such as temperature and precipitation in ~1000 pristine boreal

lakes in Europe and found that freshwater lakes in boreal regions could experience up to a 65% increase in DOC over the next 100 years. A trend of increasing DOC in boreal lakes has raised the question of how the quality and quantity of basal resources available to primary consumers such as zooplankton will change: would DOC-enriched northern freshwaters become dominated by lower quality, bacterial-source resources compared to higher quality, algal-source resources in this scenario? (Sherr & Sherr, 1988; Lennon & Cottingham, 2008;). This is an important question because the quality and type of basal resources can have cascading consequences for the transfer of essential compounds, such as essential fatty acids, to higher consumers in aquatic food webs (Arts et al. 2009).

Crustacean zooplankton are a key link in the transfer of carbon and essential compounds energy reserves in the aquatic food web from primary producers to macro-invertebrates to higher trophic levels such as fish (Thorp & Casper, 2003). Allochthonous DOC (terrestrially-produced DOC from the watershed) can, in theory, support the 'brown' pathway for zooplankton grazing, that is, it will drive a diet with a greater contribution from the bacterial community to zooplankton (Lennon & Cottingham, 2008; Sherr & Sherr, 1988). The 'green' pathway on the other hand is a diet driven by phytoplankton, and has been the long-studied trophic interaction between the base of the aquatic food web and zooplankton (Brett & Goldman, 1996). The role of the brown versus green pathway is an area of aquatic ecology that addresses nutritional quality of basal resources to primary consumers such as zooplankton. An emerging method of quantifying nutritional quality of basal

resources and their consumers is lipid biochemistry (Arts *et al.*, 2009). Lipid biochemistry analysis examines fatty acid and sterol profiles of organisms in order to trace diet composition and evaluate and organism nutritional state (Arts *et al.*, 2009).

1.2 The nutritional contribution via brown and green pathway dynamics

Increases in DOC affect basal processes of the aquatic food web by increasing levels of organic carbon uptake via the microbial community, and overall bacterial production (BP) (Jansson *et al.*, 2000; Jones, 1992). Through controlled allochthonous DOC input, experiments have shown that aquatic bacterial communities can notably contribute to the total biomass of both the benthic and pelagic zooplankton of lakes. Bacterial communities can be limited by DOC availability and are largely dependent on DOC produced as by-products via phytoplankton metabolism (i.e. extracellular DOC release) (Ask *et al.*, 2009). With terrestrial DOC input into freshwater systems, bacteria can obtain substantial amounts of dissolved carbon, and become non-limited with respect to DOC (Bergström & Jansson, 2000; Jansson *et al.*, 2000; Karlsson *et al.*, 2007). With a greater independence from phytoplankton extra-cellular DOC by-products, aquatic bacterial communities can become alternative food sources to grazing zooplankton (Hobbie *et al.*, 1999; Kankaala *et al.*, 2010; Kankaala, 1988; Rautio & Vincent, 2006). Relative bacterial contribution to diet is species dependent, as bacteria uptake can either be direct filter feeding, as in the case of many cladocerans species or indirect, for example, when heterotrophic protists (HP) consume bacteria and are themselves

consumed by copepods; this difference is based on particle size-dependent selection feeding strategies adapted by each species (Kankaala *et al.*, 2010; Karlsson *et al.*, 2007; Sommer & Sommer 2006). Pelagic freshwater bacteria have been found, thus far, to be devoid of the nutritious long chain polyunsaturated fatty acids (LC-PUFAs, > 20 carbons) and sterols compared to diatoms and cryptophytes that contain sufficient amounts to support zooplankton growth and repopulation (Arts *et al.*, 2009). HP as a food source for calanoid copepod species can infer the integration of bacteria to the diet (Breteler *et al.*, 1999).). HP grazers effectively package POM particles that are not within the direct-grazing particle range-size of calanoid copepods (Battarel *et al.*, 2005; Burkholder & Glasgow, 1997; Faithful *et al.*, 2012b). However, HP are suggested to be less essential in the non-selective grazing feeding technique used by cladocerans (Faithful *et al.*, 2012). HP abundance is predicted to increase along with BP (Vera *et al.*, 2001; Desvillettes & Bec, 2009) and can constitute a portion of zooplankton diet, and induce trophic upgrading (Breteler *et al.*, 1999), especially in calanoid copepods.

As carbon substrates and bacterial availability as an alternative food source increase, cascading effects among trophic levels in the food web can occur (Faithful *et al.*, 2012). Most studies to date that have investigated the relationship between bacterial productivity and zooplankton nutritional state have focused on single species responses in laboratory experiments. Wenzel *et al.* (2012) fed in-lab *Daphnia* diets consisting of pure algae, various level of bacteria and algae, and pure bacteria

and found that algae was a higher-quality food source that increased growth, and reproductive success in *Daphnia*. Wenzel *et al.* (2012) further examined FA profiles of the bacterial and algae food source and demonstrated that bacteria were PUFA (essential long chain FAs) depleted and algae were PUFA replete. In contrast, Breteler *et al.* (1999) found, in laboratory experiments with copepods, that a bacterial contribution to a green diet improves growth and survival. Therefore, zooplankton response to increased BP is taxon-dependant and is related to differences among taxonomic groups in direct versus indirect feeding strategies on basal resources.

Increases in DOC and BP in freshwaters can potentially have cascading consequences for the community composition and nutritional state of higher consumers in aquatic food webs. (Brett & Goldman, 1996 ; Faithful *et al.*, 2012b; Nakao *et al.*, 1999). Faithful *et al.* (2011b, 2011c), show an increase in BP can lead to increases in calanoid copepod and rotifer biomass. Taxonomic shifts such as the findings by Faithful *et al.* (2011a, 2011b) suggest shifts in energy transfer to zooplanktivores such as larval fish, and also the incorporation of the heterotrophic flagellates into the food web. Calanoid copepods are rich in PUFA (Brett *et al.*, 2009), and therefore may provide a greater transfer of PUFA to higher order organisms in ecosystems that may lack high PUFA basal resource content. PUFA and other lipids have been utilized in aquatic ecology as quantifiers of nutritional value and energy transfer among trophic levels (Arts *et al.*, 2009).

1.3 Lipids as indicators of nutritional status in aquatic ecology

Lipids play a key role in energy transfer in aquatic food webs, and are directly related to dietary nutritional quality. Polyunsaturated fatty acids (PUFA) are synthesized *de novo* at the base of the food chain and have important physiological implications for consumers at higher levels of the food chain that lack the capability to synthesize these compounds in amounts adequate to meet optimal physiological requirements (Brett *et al.*, 2006; Iverson, 2009; Parrish, 2009). Therefore, PUFA can be regarded as essential to the diet of higher order organisms, as superior predators cannot insert a double bond on the omega-3 (n-3) or omega-6 (n-6) position on a FA chain (Arts *et al.*, 2009). PUFA are the transformation of carbon into a retainable molecule by primary producers, and they can be conserved and transferred to organisms that require sufficient amounts through ingestion for optimal fitness (Gladyshev *et al.*, 2009a, b). Lipid composition is a direct way of measuring energy reserves in zooplankton and trophic transfer of PUFA through the food web (Arts *et al.*, 2009). FA composition of an organism can be variable as polar lipids (phospholipids) are conserved while neutral lipids (triglycerides, TAGs) will better reflect the diet of the consumer (Arts *et al.*, 2009; Lochmann *et al.*, 2007). FAs can be used as biomarkers, in tracing the origin of carbon in aquatic systems and its transfer between trophic levels (Arts *et al.* 2009; Iverson, 2009). Early findings of FA profiling of zooplankton species has expanded, researching in depth the importance of FAs. Modern research has advanced techniques for investigating variance in FA

body and egg composition, and focusing on essential PUFAs and what factors influence their abundance in polar and neutral lipids (Ahlgren *et al.*, 1990; Brett *et al.*, 2006; Brett *et al.*, 2009; Gladyshev *et al.*, 2009; Iverson, 2009).

In aquatic systems, two PUFA in particular have become the center of focus in terms of nutritional quality and fitness for crustacean zooplankton: eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, 22:6n3) (Arts *et al.* 2009; Sperfeld & Wacker 2012). Both PUFA are assimilated from the diet and highly retained by zooplankton (Kainz *et al.* 2004). EPA and DHA suggest higher nutritional value of zooplankton species and promote a healthy n3:n6 ratio which can lead to higher reproductive success in zooplankton (Arts *et al.*, 2009; Parrish, 2009). For example, a meta-analysis of percent DHA of total FA by Brett *et al.* (2009) showed that on average, omnivorous freshwater calanoid copepods contain 17.6 ± 9.1 % compared to 1.7 ± 1.5 % and 2.0 ± 0.9 % total FA of the whole body of herbivorous and carnivorous freshwater cladocerans, respectively; It has been suggested that a higher DHA content in copepods is attributed to their development of a more sophisticated nervous system for prey capture. Gravid cladocerans with higher levels of EPA achieve higher fecundity, maternal lipid investment, and hatching success (Sperfeld & Wacker, 2012).

Lipid reserves in crustacean zooplankton can be influenced by a wide variety of environmental and ecological factors. An early study by Arts and Sprules (1988) demonstrated that environmental stressors such as predation, can have negative

effects on lipid reserves in zooplankton, and these effects are shown in the next generation by means of reduced maternal lipid investment in eggs, decreasing offspring size. However, temperature can also influence the amount of PUFA invested into *Daphnia* eggs, as well as PUFA composition of the whole body (Sperfeld & Wacker, 2012). Moreover, the findings from Sperfeld & Wacker (2012) also found that temperature influenced lipid composition and allocation within changing temperatures; *Daphnia* eggs showed a higher TAG lipid content compared to the whole body of adult zooplankton (Sperfeld & Wacker, 2012). Examples of these studies show that environmental factors can influence lipid profiles of crustacean zooplankton, however literature is missing the effects of physico-chemical changes in freshwater, such as nutrient and DOC loading in lakes. A study by Broglio *et al.* (2003) investigated the implications of hetero- versus autotrophic protists as a prey source for marine copepods, and the implications on feeding and reproductive success. Results of this study found that food type does influence factors such as egg viability, however superiority of hetero- versus autotrophic types was not found due to PUFA content variability in each source. Similar results were also found when comparing the role of ciliates and diatoms, where food quality (amount EPA and DHA found in prey) was determined to be the limiting factors to hatching success and egg production rate in marine systems (Arendt *et al.*, 2005). Studies such as the aforementioned demonstrate that diet, much like environmental factors, can affect the lipid profile of crustacean zooplankton. The accumulation of compelling findings from these past studies has shown strong evidence that diet-derived lipids can have

ecologically significant impacts on crustacean zooplankton. PUFA can be obtained from chlorophytes, cryptophytes, and diatoms, and dinoflagellates (Brett *et al.*, 2009). As phytoplankton do not constitute the total zooplankton diet (Kankaala, 2010 ; Sherr & Sherr, 1988), the effects of bacteria and HP heavy diets on the FA profiles of zooplankton become essential in understanding possible shifts in nutritional status expected to increase with climate change factors (Larsen *et al.*, 2011). Bacterial communities are rich in 17-carbon branched FA, and lack >20-carbon PUFA which put them at a nutritional disadvantage to diatoms and cryptophytes which are PUFA rich (Arts *et al.*, 2009 ; Brett, Muller-Navarra & Persson, 2009 ; Broglio *et al.*, 2003 ; Desvillettes & Bec, 2009). This void of PUFA can be mediated through trophic upgrading of HP to produce PUFAs, and through chain elongation to create long chain FA for grazing zooplankton (Breteler *et al.*, 1999).

The objective of my MSc thesis was to test the response of crustacean zooplankton to altered basal resources in divergent resource environments that promote or suppress the green and brown food web pathways. Two types of response variables were measured: 1) ecological response in crustacean zooplankton species richness, community composition, and abundance, and 2) biochemical response in nutritional state of two major zooplankton groups with different feeding strategies, as measured by fatty acids. The study was done as field mesocosm experiment in which replicate enclosures (5000-L mesocosms) received ambient lake water (control), addition of nutrients, addition of DOC, or addition of nutrients + DOC. The following

a priori hypotheses were formulated: **H1:** nutrient addition will promote algal growth (green pathway). This will result in higher zooplankton abundance, and in higher concentrations of algal-limited essential fatty acids such as PUFAs. **H2:** DOC addition will suppress phytoplankton growth through shading but increase bacterial growth (brown pathway). This will result in lower zooplankton abundance, lower PUFA concentration, but higher concentration of bacterial fatty acids; **H3:** nutrient + DOC addition will result in an intermediate response in zooplankton abundance and PUFA content, and the highest bacterial fatty acid content since BP would be augmented by both nutrients and DOC. In reality, after analyzing the data, the artificial DOC addition in this experiment did not stimulate the brown pathway, but instead suppressed the green pathway. Therefore, results have been interpreted in light of stimulating (nutrient addition) or suppressing (DOC addition) the green pathway. My work makes a unique scientific contribution because in contrast with most other studies that have focused on single species in artificial laboratory environments, I used a community-level approach in a natural setting to understand effects of divergent resource environments on crustacean zooplankton community structure and nutritional state (+ or – green pathway). This distinction is important because my study considers consequences of differences in feeding strategies among zooplankton groups while allowing for inter-specific competitive interactions to occur that could also influence resource acquisition and relative species abundance in whole zooplankton communities. Contrasting scenarios of basal resources in freshwater ecosystems are important for conservationists to consider because they

could be produced through interactive effects of land use and climate change, and could cause cascading effects in aquatic food webs.

CHAPITRE II

2.1 The effects of basal resource manipulation on the abundance and nutritional state of freshwater crustacean zooplankton

Merante. A., Derry, A. M., Kainz, M. and Arts. M. T.

2.2 Introduction

Divergent resource pathways can be created in aquatic ecosystems through natural and anthropogenic disturbance, and can rapidly change food webs and the nutritional status of organisms (Sherr & Sherr, 1988; Ballantyne *et al.*, 2003; Lennon & Cottingham, 2008). Climate change will potentially augment terrestrial source nutrients and DOC to aquatic ecosystems, especially in association with certain land use practices such as agriculture and urbanization, through increased precipitation and terrestrial runoff. Nutrients enter lakes and rivers from watershed soils, and from agricultural and urban development as fertilizers and storm water leech into rivers and lakes (Paul & Meyer, 2001; Jeppesen *et al.*, 2009; Edmonson & Lehman, 1981). Dissolved organic carbon (DOC) in aquatic systems derives from two distinct sources: autochthonous primary production within the system or allochthonous (terrestrial) organic carbon entering the system from watershed sources (Cole *et al.*, 2002). Increases in allochthonous DOC in lakes are predicted to occur via increases

in precipitation and terrestrial runoff, changes in the duration of winter ice cover, altered thermal stratification, warmer surface waters, and changes in water chemistry (Larsen *et al.*, 2011). Nutrients and DOC have strong effects on basal resources such as phytoplankton and bacteria, which have very different nutritional value to consumers, and can therefore have divergent cascading effects among trophic levels in food webs (Alghren *et al.*, 1990; Arts *et al.*, 2009;; Brett & Goldman, 1996; Iverson, 2006). I present the first study to test how contrasting resource environments resulting from nutrient and DOC addition can impact both the community composition and nutritional state of a freshwater crustacean zooplankton community, key primary consumers in aquatic food webs.

Altered nutrients and DOC have the potential to change the nutritional state of aquatic consumers because they can strongly determine the quantity and quality of basal resources available to aquatic food webs (Andersson *et al.*, 2013; Faithfull *et al.* 2011a; Mariash *et al.* 2011; Saba *et al.*, 2011). While nutrient additions can boost algal biomass (Paul & Meyer 2001; Edmonson & Lehman, 1981) and diversify the phytoplankton community (Interlandi & Kilham, 2001), they can also cause shifts in algal quality from edible ($<35\ \mu\text{m}$) (Wagner & Kamjunke, 2011) unicellular forms to inedible filamentous algae and cyanobacteria (Schindler *et al.*, 1977). Terrestrial DOC via humic substances can reduce light penetration via shading, and cause lower algal biomass (Ask *et al.*, 2012). Both nutrients (Faithfull *et al.* 2012b) and DOC (Lennon & Cottingham, 2008; Lennon & Pfaff, 2005; Sherr & Sherr, 1988) can

potentially stimulate the bacterial community. However, the shade-suppression of phytoplankton growth in combination with enhanced bacterial growth in humic-enriched ecosystems (Faithful *et al.*, 2011b) can potentially cause food webs to have a greater dependency on bacterially derived carbon sources as a basal resource in place of phytoplankton (Faithfull *et al.*, 2011a & b; Bergström & Jansson, 1999; Jansson *et al.*, 2000). In fact, there is evidence that increased terrestrial inputs of DOC to aquatic ecosystems can lead to reduced production in higher trophic levels (Karlsson *et al.*, 2009) and overall net ecosystem production (Ask *et al.*, 2012). These studies are suggestive that differences in the quantity and quality of basal resources resulting from terrestrial additions of nutrients and/or humic substances could possibly affect how essential compounds are transferred up aquatic food webs to higher trophic levels.

Zooplankton provide a key trophic link between basal resources associated with algae and bacteria, and higher consumers such as predatory invertebrates and fish (Iverson *et al.*, 2004). As such, these organisms can provide information about how terrestrial runoff could affect the transfer of essential compounds from basal resources to higher trophic levels (Ballantyne *et al.*, 2003). Lipids or fatty acids have emerged as one of the most powerful food web markers for assessing sources and nutritional quality in aquatic ecosystems (Arts *et al.*, 2009; Muller-Navarra *et al.*, 2005; Iverson *et al.*, 2004). PUFA such as the omega-3 (n-3) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) positively influence development (Arts *et*

al., 2009) and fecundity (Arendt *et al.*, 2005), respectively, and are synthesized *de novo* at the base of the food web in phytoplankton (Arts *et al.*, 2009 ; Sperfeld & Wacker, 2012). EPA and DHA, along with other PUFA, are then transferred up the aquatic food web via dietary accumulation (Gladyshev *et al.*, 2009). Higher order organisms can create the DHA and EPA, through elongation of the PUFA alpha-linolenic acid (ALA), however this is done with low efficiency (Arts *et al.*, 2009). PUFA, such as DHA and EPA, can be regarded as essential as they are required through dietary consumption to meet a higher order organisms biological needs (Arts *et al.*, 2009). Any environmental parameter that affects the distribution of PUFA at the base of the food chain could alter the transfer of these compounds to consumers in aquatic ecosystems (Taipale *et al.*, 2011; Fuschino *et al.*, 2011; Arts *et al.*, 2009). Terrestrial inputs of nutrients and/or DOC could therefore potentially alter the availability of essential fatty acids to intermediate aquatic consumers such as zooplankton by stimulating or suppressing phytoplankton growth.

Crustacean zooplankton carry different amounts of specific PUFA depending on taxonomic affiliation (at the level of Order), and even from species to species (Sperfeld & Wacker, 2012; Sekino *et al.*, 1997). This is possibly because of differences in the dietary intake of phytoplankton species, and evolution of life history traits (Arts *et al.*, 2009). For example, copepods are known to be comprised of relatively more DHA compared to cladocerans, which contain relatively more EPA (Arts *et al.*, 2009). These groups have different functional feeding roles in ecosystems

because cladocerans are generally passive filtration feeders (Sastri *et al.*, 2011) and copepods actively select their food (Kleppel, 1993). Therefore, it is important to consider both community composition and fatty acid (FA) when assessing responses in nutritional state of crustacean zooplankton to terrestrial runoff.

There is a historical and ongoing focus on community and trophic food web responses to nutrients (Schindler *et al.*, 1977; Faithful *et al.* 2011b), and a more recent surge of interest to understand the ecological consequences of increased allochthonous DOC to freshwater (Pace *et al.* 2004; Rautio and Vincent 2006; Karlsson *et al.* 2009; Tranvik *et al.*, 2009; Brett *et al.* 2009; Lennon *et al.*, 2013) and marine (Anderson *et al.* 2013) ecosystems. Of recent studies that have addressed the effects of allochthonous DOC on aquatic foods, most of the focus has been on bacteria and phytoplankton communities (Jansson *et al.* 2008; Anderson *et al.*, 2013), and ecological responses of zooplankton are not well understood (Nicolle *et al.*, 2012; Mitrovic *et al.*, accepted Journal of Plankton Research). Divergent resource environments associated with nutrients and allochthonous DOC could not only affect community composition and structure, but also the nutritional state of the zooplankton through changes in quality and quantity of basal resources at the bottom of the food web. However, no study to date has addressed changes in the nutritional state of crustacean zooplankton related to increases in DOC in freshwater ecosystems. Moreover, of studies that have addressed zooplankton fatty acid response to algal versus bacterial diets, most have focused on single species manipulations in

laboratory experiments (Breteler et al. 1999; Wenzel et al. 2012). However, it is important to consider responses of zooplankton in a community context because feeding strategies differ between cladocerans and copepods, and because inter-specific competition can influence resource acquisition and relative species abundance in whole zooplankton communities.

My work makes a unique scientific contribution because unlike most other studies that have focused on single species in artificial laboratory environments, I used a community-level approach in a more natural setting (5000-L *in situ* field mesocosm enclosures) to understand effects of divergent resource environments on crustacean zooplankton community structure and nutritional state (+ or – green pathway related to nutrient and/or DOC addition). The following *a priori* hypotheses were formulated: **H1**: nutrient addition will promote algal growth (green pathway). This will result in higher zooplankton abundance, and in higher concentrations of algal-limited essential fatty acids such as PUFAs. **H2**: DOC addition will suppress phytoplankton growth through shading but increase bacterial growth (brown pathway). This will result in lower zooplankton abundance, lower PUFA concentration, but higher concentration of bacterial fatty acids; **H3**: nutrient + DOC addition will result in an intermediate response in zooplankton abundance and PUFA content, and the highest bacterial fatty acid content since BP would be augmented by both nutrients and DOC. Contrasting scenarios of basal resources in freshwater ecosystems are important for conservationists to consider because they could be

produced through interactive effects of land use and climate change, and could cause cascading effects in aquatic food webs.

I conducted a field mesocosm experiment to assess how divergent resource pathways related to nutrient and DOC addition (nutrients, humic substances, and nutrients + humic substances) could impact the abundance, composition, and nutritional state of a crustacean zooplankton community. I predicted that nutrient addition would boost phytoplankton growth and increase PUFA content of crustacean zooplankton lipid profiles, but that it would also possibly come with the compromise of higher abundance of less edible phytoplankton that are a poorer source of PUFA. I anticipated that humic addition would suppress phytoplankton quantity and quality, and light-limit the production of PUFA from high quality phytoplankton. With the suppression of a green-pathway, bacteria may play a larger role in the dietary contribution of crustacean zooplankton, impairing nutritional state. The shading effect of humic material in combination with nutrients addition was predicted to suppress any positive effects of nutrients for algal growth, and result in low zooplankton abundance and poor nutritional state, similar to the addition of humic material alone. Community responses of crustacean zooplankton, and their ability to accumulate PUFA in face of environmental change has proven critical for understanding of nutritional quality of aquatic biota at higher levels in aquatic food webs (Arts *et al.*, 2009; Lochmann *et al.*, 2007; Brett *et al.*, 2006;).

2.3 Methodology

2.3.1 Field Mesocosms

Experimental treatments were established as a factorial design in 24 *in-situ* polyethylene mesocosms in Lake Cromwell, Quebec, Canada (45°59' N 73°59' W). Lake Cromwell is neutral (pH 7.2), oligo-mesotrophic ($7.91 \mu\text{g P L}^{-1}$) freshwater lake with relatively low algal biomass ($1.99 \mu\text{g chl}a \text{ L}^{-1}$), moderate water transparency (Secchi depth of 3.5 m), moderate dissolved organic carbon (DOC) concentration of 4.68 mg L^{-1} and conductivity of $20.3 \mu\text{S cm}^{-1}$ (unpublished data, Derry). The mesocosms (1 m diameter, 6 m depth) were filled with 54- μm - filtered epilimnetic water from Lake Cromwell to a volume of approximately 5000 L. I established two sets of four treatments ($n=3$ replicates; total 24 mesocosms) such that the duration of the experiment was 3 weeks for 12 of the enclosures, and 6 weeks for the other half of the enclosures. Each set of enclosures (week 3 and week 6 duration) received the same handling and experimental manipulation in terms of nutrient and humic addition, zooplankton stocking, and sampling for water chemistry and response variables. Experimental treatments ($n=3$ replicates) were comprised of: 1) a nutrient addition, 2) a humic addition, 3) a nutrient + humic addition, and 4) ambient Lake Cromwell water as reference for the experimental contrasts (Figure 1). Nutrient additions were achieved by single addition of KH_2PO_4 and NaNO_3 to raise the TP concentration from $7.9 \mu\text{g L}^{-1}$ to $80.55 \pm 1.8 \mu\text{g L}^{-1}$ and the TN concentration from 0.29 to 0.465 ± 0.03 ppm. Humic treatments were created via a single addition of an agricultural additive SuperHume® derived from peat moss extract

(<http://www.earthcrew.com/SuperHume.htm>). Humic treatments raised the DOC concentration from 4.7 mg L^{-1} to $12 \pm 1.3 \text{ mg L}^{-1}$ to mimic high DOC levels of the surrounding region (Del Giorgio, unpublished). Nutrient + humic treatments reached concentrations of $56 \pm 14.6 \text{ } \mu\text{g L}^{-1}$ TP, $0.61 \pm 0.13 \text{ ppm}$ TN, and $12 \pm 4.3 \text{ mg L}^{-1}$ DOC. The reference enclosures reflected general conditions of Lake Cromwell ([TP] = $7.4 \text{ } \mu\text{g L}^{-1}$, TN = 0.29 ppm ; [DOC] = 5.7 mg L^{-1}). The mesocosms were allowed to equilibrate for one week to allow phytoplankton and microbial communities to regain abundance following mesocosm water filling. After one week, the mesocosms were stocked with zooplankton from Lake Cromwell at concentrations equivalent to those found in the water column during the day. The zooplankton were collected via $54\text{-}\mu\text{m}$ Nitex nets of 30 cm diameter and 1 m in length, and immediately released into mesocosm after each haul. Experimental design was set up to ensure that intensive sampling of organisms for lipids at weeks 3 and 6 would not deplete or alter the experimental zooplankton communities.

2.3.2 Physico-chemical conditions

Physico-chemical conditions of each of the enclosures were measured once a week for the following parameters: photosynthetically active radiation (PAR), dissolved organic carbon (DOC), total phosphorus (TP), and total nitrogen (TN) at 0.5 m. A multiparameter YSI Pro Plus probe (model 10102030; Yellow Springs Inc.) was used to collect temperature (T), dissolved oxygen (DO), pH, and conductivity (C) at 0.5 m depth per mesocosm. PAR was measured using a LI-COR® LI-190

Quantum Sensor at 0.5 m depth per mesocosm. Secchi depth, primary production and primary respiration were measured at 3 discrete time intervals during the experiment. TP was quantified by spectrometry on a Biochrom® Ultrospec 2100 pro.A (Cambridge, England). Spectrophotometric analysis of TP was done by the molybdenum-blue method following persulfate digestion. TN analysis was conducted as NO_3^- using a Biochrom® Alpkem Flow solution IV autoanalyzer (Cambridge, England) following alkaline persulfate digestion (Wetzel & Likens, 2000). DOC was measured in 0.45 μm -filtered water by wet oxidation with an O.I.® Analytical Total Carbon Analyzer (College Station, Texas).

2.3.3 Basal Resources: Phytoplankton and Bacteria

Algal responses to the mesocosm treatments were measured by means of chlorophyll *a* (Chl *a*) as an estimate of algal biomass at weeks 1, 3, and 6 as well as gross primary production (PPr; $\mu\text{M O}_2 \text{ h}^{-1}$) at weeks 1, 2, and 6. Respiration (R) rates were measured in all 12 mesocosms during week 2 by 24-h, *in situ* incubations of unfiltered water in 4-L cubitainers. Initial and final samples of DO were collected and measured by membrane inlet mass spectrometry following Bouvier & del Giorgio (2002). Isotopic ratios of $^{18}\text{O}:^{16}\text{O}$ (for H_2O and O_2) are reported relative to Vienna standard mean ocean water (VSMOW) using standard δ notation. Samples for $^{18}\text{O}-\text{O}_2$ were analyzed at the University of Ottawa Stable Isotopes Laboratory. Week 2 respiration (R), $^{18}\text{O}-\text{O}_2$, and $^{18}\text{O}-\text{H}_2\text{O}$ were used to calculate gross primary production (PPr) and P:R following Quinonez-Rivera et al. (2007). To determine the

taxonomic composition of phytoplankton present in the enclosures, algal samples were collected via a 6 m water integration tube at concentrations present in the enclosures, fixed with Lugol's solution, and stored in the dark. Phytoplankton composition was identified to genus and was quantified via microscopy for the midpoint of the experiment's duration (inverted microscope, Olympus IX71, Olympus Japan). Phytoplankton composition at week 3 data is presented in Appendix 1. Bacterial production (BP) measurements were done using the 3H-leucine incorporation technique (Kirchman, 1993), following the protocol described in del Giorgio *et al.* (2006).

2.3.4 Crustacean Zooplankton Communities

Zooplankton communities were sampled each week with a 54 μm Nitex Wisconsin net (diameter of 15 cm) from the entire water column within each of the enclosures. Zooplankton were anaesthetized with carbon dioxide and preserved with a 4% sugar-formalin mixture. Minimums of 250 individuals were counted via microscopy (MI- SZ Z-IL-ST Olympus stereomicroscope, Japan). Only adult individuals were enumerated, and were identified to species level, with the exception of *Chydorus spp.* following the "Guide to Zooplankton of the Great Lakes" (Balcer *et al.*, 1984) and a previous inventory of zooplankton species of Lake Cromwell (Beisner, unpublished). While samples were counted for every week of the experiment, only the zooplankton concentrations at weeks 3 and 6, and in Lake Cromwell at the onset of the experiment are shown for comparison with fatty acids.

2.3.5 Crustacean Zooplankton Fatty Acids

Crustacean zooplankton were intensively sampled from mesocosms at the end of each experimental trial (week 3 and week 6). Crustacean zooplankton were individually sorted into major taxonomic groups (cladocerans, calanoid copepods) for fatty acid analyses using SZ- 2-IL-ST Olympus stereomicroscopes. I excluded cyclopoid copepods from lipid analyses because to their omnivorous and complex diet (Wiujamson, 1984) that would have made the interpretation of lipid profiles ambiguous. Crustacean zooplankton samples were immediately frozen at -80°C with liquid nitrogen, stored at UQÀM, and then freeze-dried. Total lipids of organisms were extracted using chloroform:methanol (2:1) methodology (Heissenberger *et al.* 2010) and subsequently separated into polar and neutral lipids. All samples were derivatized to fatty acid methyl esters (FAME) and analyzed on a gas chromatograph (TRACE GC THERMO, Detector: FID 260°C , Carrier gas: H_2 : 40 mL min^{-1} , N_2 : 45 mL min^{-1} , air: 450 mL/min , temperature ramp: $140^{\circ}\text{C}(5\text{min})-4^{\circ}\text{C/min}-240^{\circ}\text{C}(20\text{min})=50\text{ min}$) equipped with a temperature-programmable injector and an autosampler. A SupelcoTM SP-2560 column (100 m, 25 mm i.d., 0.2 μm film thickness) was used for FAME separation. Excalibur 1.4TM was used for calculation and, if necessary, manual resetting of the chromatograms. I focused on non-polar, triglyceride (TAG) FAs in crustacean zooplankton because they represent dietary-based energy reserves (Arts *et al.*, 2009). Fatty acid concentrations were calculated using calibration curves based on known standard concentrations. All analyses were

carried out at the Wasser-Cluster (Lunz-am See, Austria) analytical laboratories led by M. Kainz.

2.3.6 Statistical Analyses

All physico-chemical parameters (PAR, DOC, TN, TP, pH, and DO) and basal resource measurements (Chl *a*, Gross PPr, BP, and Respiration) were analyzed using factorial ANOVA detecting for effects of treatment, week and treatment-week interaction. Any significant differences ($p < 0.05$) detected among experimental treatments in the factorial ANOVA were followed by Tukey HSD post-hoc tests. All physico-chemical parameters were subject to factorial ANOVA were tested for normality under the Shapiro-Wilk's tests and equal variance under the Levene's test (*STATISTICA* 11.0, StatSoft). Data was log transformed ($\text{Log}(x)$) to meet ANOVA assumptions if assumption were not met by untransformed data (Appendix 1). No significant differences were found between physico-chemical parameters between mesocosms of 3 and 6 week life spans at each measured point in time (RM -ANOVA; Table 1, Tukey's Test, $p > 0.05$) (Appendix 1), therefore only physico-chemical and basal resource data is shown from week 6 mesocosms.

Crustacean zooplankton biomass was calculated from species-specific average body lengths drawn from past literature for species present in our study. Total zooplankton community biomass and biomass of major taxonomic groups (calanoid and cyclopoid copepods and cladocerans) per m^3 were calculated by using standard regression for biomass calculation from Culver *et al.*, 1985.

Crustacean zooplankton biomass was analyzed by factorial ANOVA to test for significant differences among and within treatments. Factorial ANOVA assumptions of normality under the Shapiro-Wilk's tests and equal variance under the Levene's test (*STATISTICA* 11.0, StatSoft) were met. Factorial ANOVA were followed by Tukey HSD post-hoc tests for significant ($p < 0.05$) effects of treatment, week, and treatment-week interaction.

Crustacean zooplankton TAG FA profiles were analyzed using factorial ANOVA, to test for FA loss, gain, or retention among experimental treatments. FA profiles were expressed as ratios, wherein specific FA groups were represented as percentages of total TAG FAs. Factorial ANOVA were run on omega-3 (n=3), omega-6 (n=6) and bacterial TAG FA of calanoid copepods and cladocerans. FA profiles subject to ANOVA were tested for normality under the Shapiro-Wilk's tests and equal variance under the Levene's test (*STATISTICA* 11.0, StatSoft); replication was unequal among treatments. All statistical tests of variance on crustacean zooplankton FA profiles were subject to a Tukey's post-hoc test when significance was detected in the ANOVA. Significance was reported at $p < 0.05$. FA profile replication was reduced in environments such as humic (n=2) and nutrients + humic (n=2) treatments at weeks 3 and 6, as zooplankton tissue was limited, however standard error does not exceed $\pm 4\%$ among 3 replicates.

2.4 Results

2.4.1 Water treatment

Distinct water treatments were created by addition of nutrients, humic substance, and nutrients + humic substance (Factorial ANOVA, treatment Table 1). The target concentrations of DOC ($>12 \text{ mg L}^{-1}$) were achieved in mesocosms with humic and nutrient + humic treatments, and these concentrations were higher than in the control and nutrient mesocosms (Factorial ANOVA, water treatment effect, Tukey's Test, Table 1; Fig. 1A). DOC was significantly higher in the nutrient + humic treatment (Factorial ANOVA; Tukey's Test, $p < 0.005$) (Fig. 1A) compared to our other water treatments; DOC levels in our humic treatment were marginally insignificant (Factorial ANOVA; Tukey's Test, $p = 0.055$) (Fig. 1A) compared to our other water treatments. Shading was apparent in humic and nutrient humic treatments, which was indicated by reduced light regression coefficients ($<1.25 \text{ K m}^{-1}$) in humic and nutrient + humic mesocosms (Factorial ANOVA, water treatment, Table 1; Fig. 1B). Significant higher shading was found in our humic (Factorial ANOVA; Tukey's Test, $P < 0.05$) and nutrient + humic treatments (Factorial ANOVA; Tukey's Test, $p < 0.05$) (Table 1, Fig. 1B). Phosphorus concentrations ($>55 \text{ mg L}^{-1} \text{ TP}$) were higher in nutrient and nutrient + humic mesocosms than in mesocosms without nutrient addition (Factorial ANOVA, water treatment, Table 1) (Fig. 1C); Nitrogen levels did not differ among treatments (Table 1) (Fig. 1D). TP was significantly higher in the nutrient (Factorial ANOVA; Tukey's Test, $p < 0.005$) and nutrient + humic treatments (ANOVA; Tukey's Test, $p < 0.005$) compared to the control and humic treatments

(Fig. 1C). Nutrients and humic substances, measured by DOC, TP, and TN, were lower in week 6 compared to week 3 (Factorial ANOVA, week effect, Table 1; Fig. 1A, C, D). Week was insignificant for all other physico-chemical variables (Factorial ANOVA; Table 1, Tukey's Test, $p>0.05$).

2.4.2 Basal Resources: Phytoplankton and Bacteria

Differences in basal resources (algae and bacteria) among water treatments were detected (Factorial ANOVA, treatment effect, Table 1). Algal biomass, as estimated by chlorophyll *a*, was higher in nutrient (Factorial ANOVA, Tukey's Test, $p<0.005$) and nutrient +humic water treatments (Factorial ANOVA, Tukey's Test, $p<0.005$) and bacterial production was higher in our nutrient (Factorial ANOVA, Tukey's Test, $p<0.05$) and nutrient + humic treatments (Factorial ANOVA, Tukey's Test, $p<0.05$). Gross PPr was also found to be significantly different among treatments (Factorial ANOVA, treatment effect, Table 1). Higher Gross PPr was found in our nutrient treatment (Factorial ANOVA, Tukey's Test, $p<0.001$) and lowest in our humic treatment. No significant differences were found between basal resource parameters between mesocosms of 3 and 6 week life spans (Factorial ANOVA; Tukey's Test, $p>0.05$). No significant differences were seen between results of 3 and 6 week trends of basal resource data (Factorial ANOVA; Tukey's Test, $p>0.05$).

Chl-*a* was highest in our nutrient treatment (Factorial ANOVA; Tukey's Test, $p < 0.0005$) and our nutrient + humic treatment (Factorial ANOVA; Tukey's Test, $p < 0.0005$) (Fig. 2A). BP was similarly highest in our nutrient treatment (Factorial ANOVA; Tukey's Test, $p < 0.0005$) and our nutrient + humic treatment (Factorial ANOVA; Tukey's Test, $p < 0.0005$) (Fig. 2B); BP did not significantly differ between our humic and control treatments (Factorial ANOVA; Tukey's Test, $p > 0.05$) (Fig. 2B). pH (Factorial ANOVA; Tukey's Test, $p < 0.0005$) and DO (ANOVA; Tukey's Test, $p < 0.0005$) were highest in the nutrient treatments suggesting high photosynthetic activity, but were non-significant among the other three treatments (Fig. 2C, D). Significantly higher gross PPR (Fig. 3A) were found in our nutrients treatments (Factorial ANOVA; Tukey's Test, $p < 0.05$).

Table 1. Results of factorial ANOVA for mesocosm water treatment physico-chemical and basal resource parameters among mesocosm treatments (n=4). Results of significance ($p < 0.05$) are bolded, and results of non-significance ($p > 0.05$) are denoted by ‘-’.

	Physico-chemical parameters						Basal resource parameters			
	DOC	K	TP	TN	pH	DO	Chl <i>a</i>	BP	PPr	Respiration
Df effect	3	3	3	3	3	3	3	3	3	3
Df Error	48	16	48	42	32	32	17	32	24	24
Water Treatment										
F	36.605	15.118	35.407	--	29.55	67.0	17.135	33.910	56.342	83.305
P	<0.001	<0.001	<0.001	--	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Week										
F	4.778	--	10.422	3.767	5.8	35.3	--	8.541	--	--
P	<0.05	--	<0.001	<0.01	<0.005	<0.001	--	<0.001	--	--
Treatment*Week										
F	--	--	--	2.803	7.82	4.1	--	2.545	--	--
P	--	--	--	<0.005	<0.001	<0.005	--	<0.01	--	--

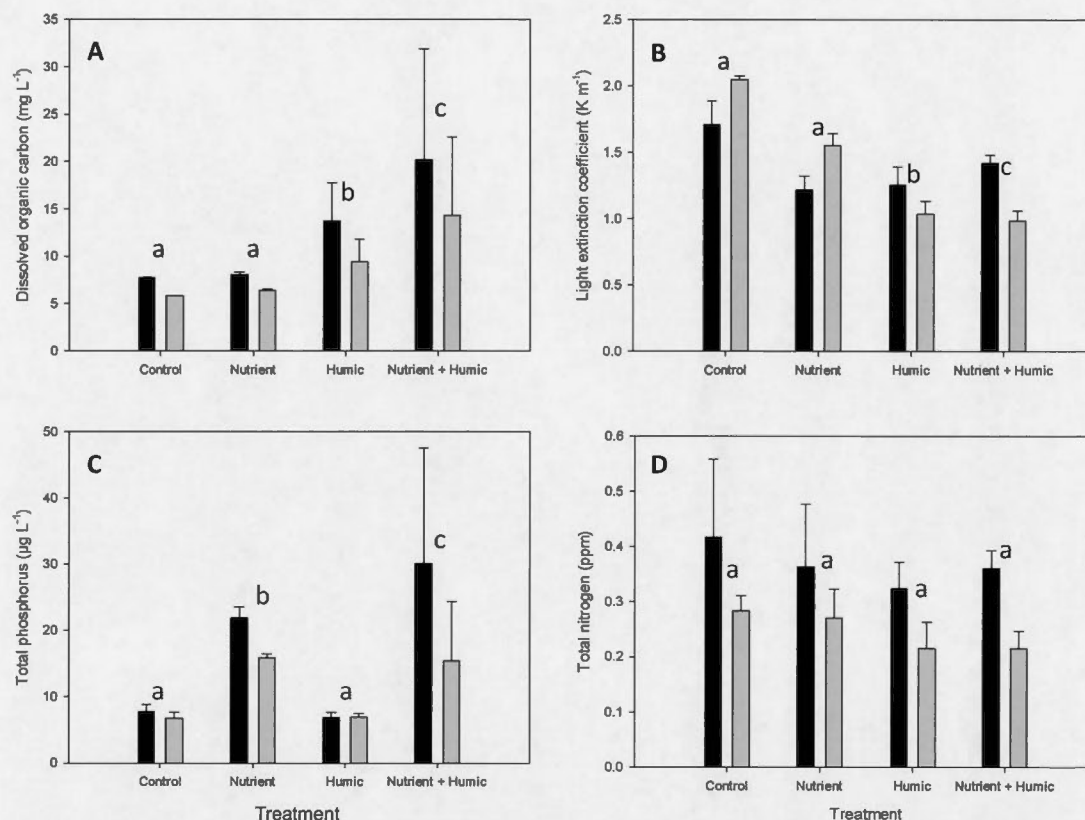


Figure 1. Physico-chemical parameters A) DOC (mean $\mu\text{g per L}^3 \pm \text{SE}$), B) the light regression coefficient (mean $\text{K per m}^3 \pm \text{SE}$), C) TP (mean $\mu\text{g per L}^3 \pm \text{SE}$), and D) TN (mean ppm) $\pm \text{SE}$ during week 3 (black) and week 6 (grey) of mesocosms. Water treatments shown only depict those mesocosm with a 6 week lifespan. Results of post-hoc Tukey's test across treatments at week 6 are indicated on figure by letters above bars.

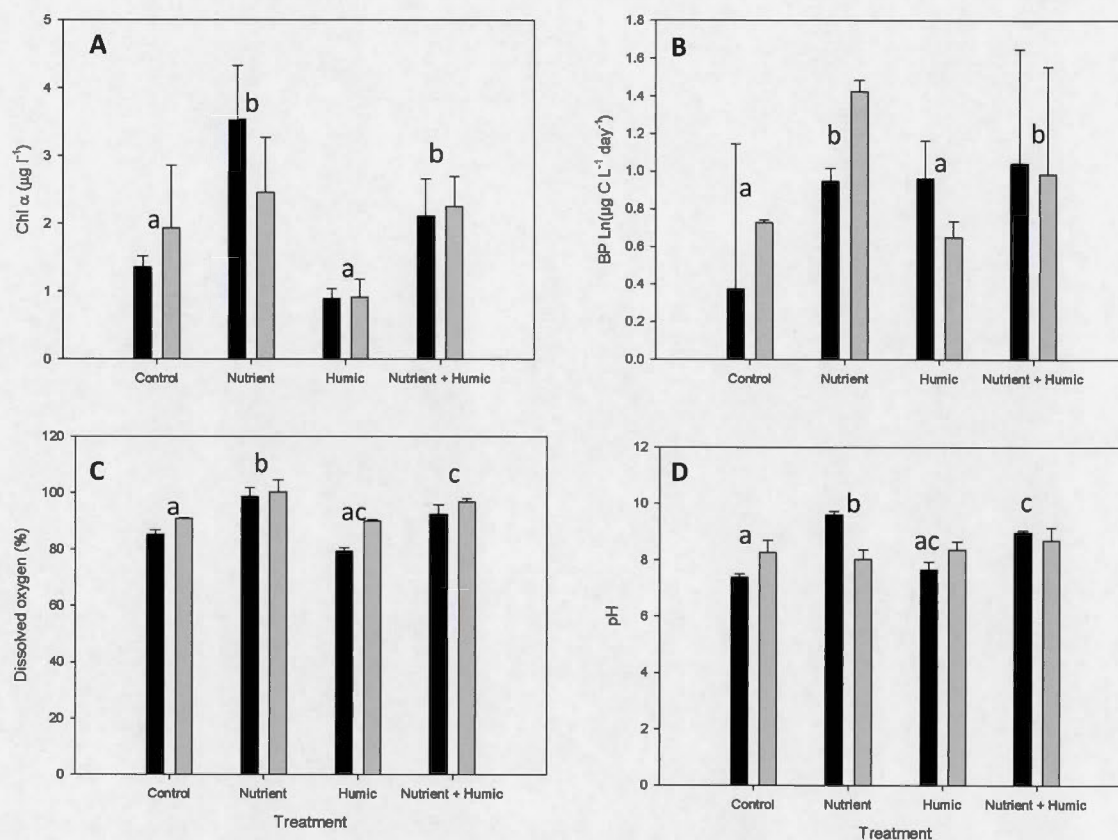


Figure 2. Basal resource (A,B) and physico-chemical (C, D) parameters A) Chl *a* (mean $\mu\text{g per L}^3 \pm \text{SE}$), B) BP (mean $\mu\text{g C per L}^3 \text{ per day} \pm \text{SE}$), and *proxim*-parameters to basal resources C) DO (mean $\% \pm \text{SE}$), and D) pH (mean $\pm \text{SE}$), during week 3 (black) and week 6 (grey) of mesocosms. Water treatments shown only depict those mesocosm with a 6 week lifespan. Results of post-hoc Tukey's test across treatments at week 6 are indicated on figure by letters above bars.

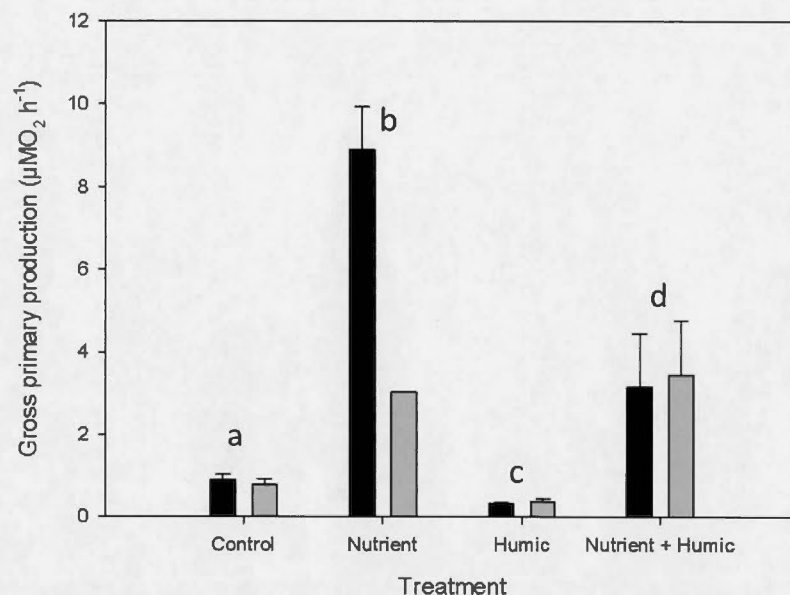


Figure 3. Basal resource parameter Gross PPr (mean μMO_2 per hour) \pm SE during week 3 (black) and week 6 (grey) of mesocosms. Water treatments shown only depict those mesocosm with a 6 week lifespan. Results of post-hoc Tukey's test across treatments at week 6 are indicated on figure by letters above bars.

2.4.3 Crustacean zooplankton community response

Both water treatment and week had significant main and interactive effects on the zooplankton community of Cromwell Lake. Shannon-Weiner diversity was reduced in our nutrient + humic treatments (Factorial ANOVA; Water Treatment main effect, Table 2, Tukey's Test, $p < 0.001$), whilst our control, nutrient, and humic treatments did not significantly differ from one another. Total abundance (TA) was also reduced in humic and nutrient + humic treatments for the major crustacean zooplankton orders (Factorial ANOVA; Table 2, water treatment main effect; Tukey's Test, $p < 0.001$)

With the exception of the cyclopoid copepod *Acanthocyclops robustus*, all crustacean zooplankton species abundances were reduced by humic and nutrient + humic treatments (Factorial ANOVA, Table 2, water treatment main effect, Tukey's Test, $p < 0.001$). With the exception of the calanoid copepod *Leptodiaptomus minutus*, and the cyclopoid copepod *A.robustus* the week at which mesocosms were sampled was significant for the abundance of all other crustacean zooplankton species (Factorial ANOVA, Table 2, week main effect Tukey's Test, $p < 0.01$). Interactions between water treatment and week were present only for biomass of major crustacean zooplankton orders and the abundance of the cladoceran species *H. gibberum* (Factorial ANOVA, Table 2, treatment x week effect, Tukey's Test, $p < 0.001$). This was because by week 6, *gibberum* no longer persisted in our mesocosms.

Total crustacean zooplankton biomass was affected by water (Table 3, Factorial ANOVA, Table 3). Highest biomasses were found in our nutrient treatments (Table 3, Factorial ANOVA, treatment main effect, Tukey's Test, $p < 0.001$) and lowest in our humic treatments (Table 3, Factorial ANOVA, treatment main effect, Tukey's Test, $p < 0.001$).

Table 2. Results of factorial ANOVA for crustacean zooplankton community structure and composition among mesocosm treatments ($n=4$), wherein Shannon-Weiner (S-W) diversity index and total abundance (TA) of the community are presented. Results of significance ($p<0.05$) are bolded, and results of non-significance ($p>0.05$) are denoted by ‘-’.

Crustacean zooplankton community response											
Df effect	S-W diversity	TA		TA		TA		TA		TA	
		Calanoid copepods	Cladocerans	<i>D. ambigua</i>	<i>D. longirimis</i>	<i>S. Lieder</i>	<i>H. gibberum</i>	Cyclopoid	<i>C. scifer</i>	<i>A. robustus</i>	TA
3	3	3	3	3	3	3	3	3	3	3	3
Df Error	32	32	32	32	32	32	32	32	32	32	32
Water											
Treatment											
F	7.857	59.288	51.55	16.665	25.207	11.668	18.287	12.11	4.803	--	--
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	--	--
Week											
F	7.031	--	3.413	18.336	4.804	7.901	132.659	12.208	8.098	--	--
P	<0.001	--	<0.05	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	--	--
Treatment											
*Week											
F	--	4.627	3.257	--	--	--	7.823	3.167	--	--	--
P	--	<0.001	<0.05	--	--	--	<0.001	<0.01	--	--	--

Within the zooplankton order of calanoid copepods, only one species was present in the community – *L. minutus*. This species was most abundant in nutrient-enriched mesocosms compared to the control (Table 2, Fig. 4A, $p < 0.001$, Tukey's Test), and the most reduced in the humic treatment compared to the control (Table 2, Fig. 4A, $p < 0.001$, Tukey's Test). The abundances of this copepod were not significantly affected by the nutrient + humic treatment compared to the control, and no differences were detected within treatments between week 3 and 6. Because this order was comprised of a single species, calanoid biomass results followed the same pattern as we report here for *L. minutus* abundance (Fig. 4B, $p < 0.001$, Tukey's Test).

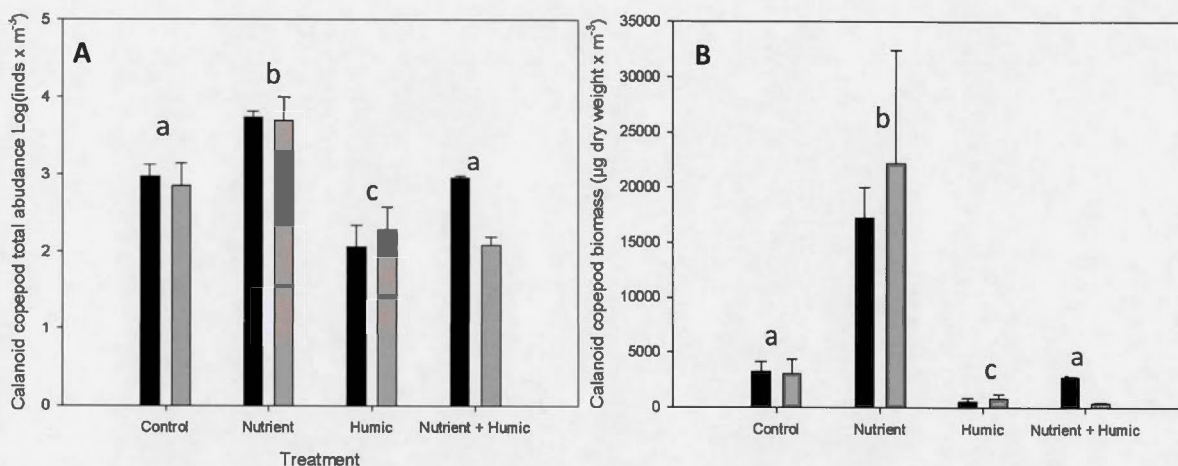


Figure 4. A) Calanoid copepod (*L. minutus*) total abundance (mean Log(individuals per m³) \pm SE) and B) calanoid copepod (*L. minutus*) biomass (mean μ g dry weight per m³) \pm SE) during week 3 (black) and week 6 (grey) of mesocosms. The results of post-hoc tests among treatments are indicated. Results of post-hoc Tukey's test across treatments are indicated on figure by letters above bars.

Cladoceran (*D. ambigua*, *D. longiremis*, *S. linder*) total abundances ranged from 1360 to 90 individuals m⁻³. Significantly higher total abundances of cladocerans were found in our nutrient treatments (Fig. 5A, $p < 0.001$, Tukey's Test) whilst significantly lower total abundances were found in our humic treatment (Fig. 5A, $p < 0.001$, Tukey's Test) and nutrient + humic treatment (Fig. 5A, $p < 0.05$, Tukey's Test) when compared to our control treatment. There were no significant differences within treatments between week 3 and 6.

Cladoceran biomass showed similar results. Highest cladoceran biomass was found in our nutrient treatments (Fig. 5B, $p < 0.001$, Tukey's Test) and the lowest biomass was found in our humic treatment (Fig. 5B, $p < 0.001$, Tukey's Test) when

compared to the control treatment; no significant differences were seen between our nutrient + humic and control treatments. Week of sampling showed a significant effect on cladoceran biomass among treatments (Table 3, Factorial ANOVA, week effect), a significant interaction was found between time and conditions (Table 3, Factorial ANOVA, treatment x week effect). There were no significant differences within treatments between week 3 and 6.

Cladoceran species total abundance varied among treatments. *D. ambigua* and *D. longiremis* showed significantly higher total abundances within our nutrient treatment (Fig. 6A, B, $p < 0.01$, Tukey's Test) and lower total abundances within our humic treatment (Fig. 6A, B, $p < 0.05$) when compared to our control treatment; nutrient + humic and control treatments showed no significant differences in total *D. ambigua* and *D. longiremis* abundance. *S. lieder* had significant decreases in total abundance in our humic (Fig. 6C, $p < 0.05$, Tukey's Test) and nutrient + humic (Fig. 6C, $p < 0.05$, Tukey's Test) treatments compared our control; no significant differences were found between *S. lieder* total abundances when compared to our control. From weeks 1 to 3, *H. gibberum* total abundance was significantly higher in our nutrient treatment (Fig. 6D, $p < 0.05$, Tukey's Test), and significantly lower in our humic (Fig. 6D, $p < 0.05$, Tukey's Test) and nutrient + humic treatments when compared to our control. There were no significant differences within treatments between week 3 and 6. From weeks 4 to 6, *H. gibberum* diminished and was not present.

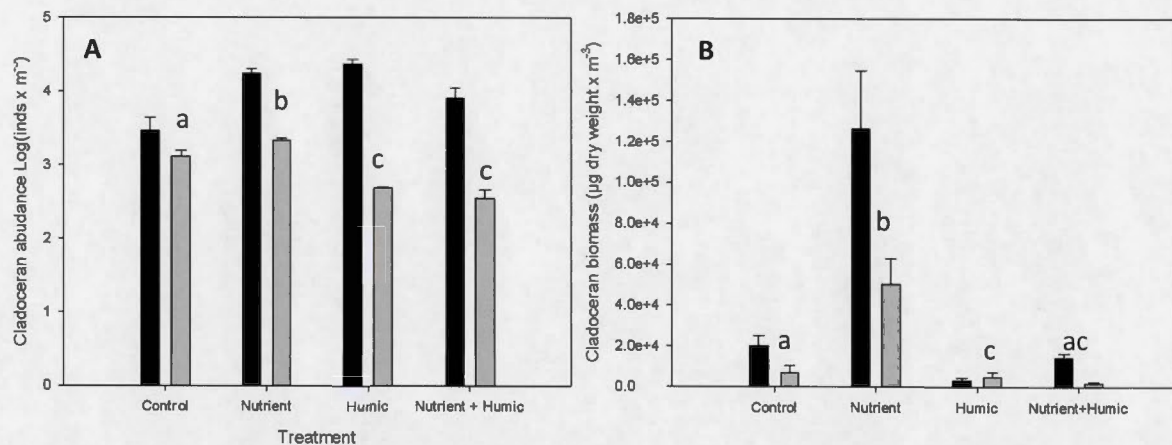


Figure 5. A) Cladoceran (*D. ambigua*, *D. longiremis*, and *S. lieder*) total abundance (mean Log(individuals per m³) \pm SE) and B) cladoceran (*D. ambigua*, *D. longiremis*, and *S. lieder*) biomass (mean μ g dry weight per m³) \pm SE) during week 3 (black) and week 6 (grey) of mesocosms. Results of post-hoc Tukey's test across treatments are indicated on figure by letters above bars.

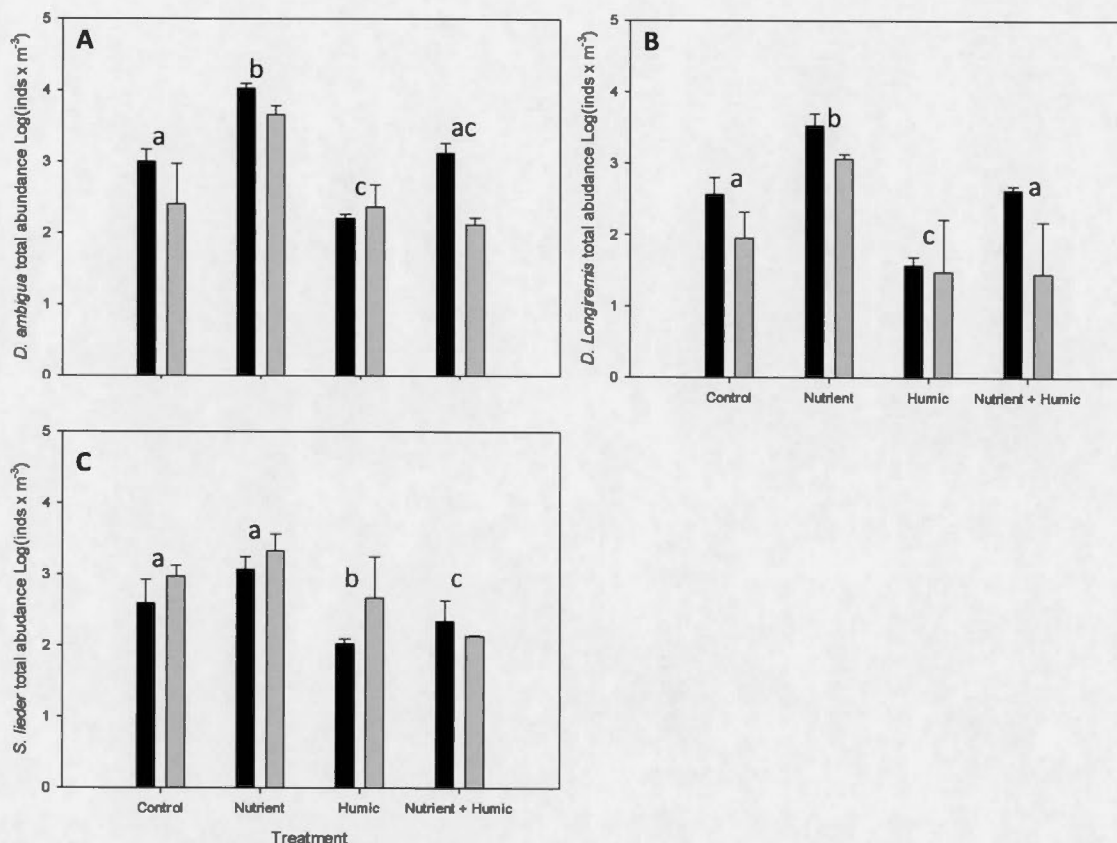


Figure 6. A) The cladoceran *D. ambigua*, B) *D. longiremis*, and C) *S. lieder* total abundance (mean Log(individuals per m³) \pm SE) during week 3 (black) and week 6 (grey) of mesocosms. Results of post-hoc Tukey's test across treatments are indicated on figure by letters above bars.

Cyclopoid copepod community response

Cyclopoid copepods (*C. scutifer*, *A. robustus*) total abundances ranged from 7735 to 89 individuals m⁻³. Significantly higher total abundances of cyclopoid copepods were found in our nutrient treatments (Fig. 7A, $p < 0.001$, Tukey's Test) when compared to our control treatment. No significant differences were found

when comparing our humic and nutrient + humic treatments to our control. There were no significant differences within treatments between week 3 and 6.

The lowest cyclopoid biomass was found in our nutrient + humic treatment (Fig. 7B, $p < 0.001$, Tukey's Test) when compared to the control treatment; no significant differences were seen between our nutrient or humic and control treatments when compared to the control. Week of sampling showed a significant effect on cyclopoid copepod biomass among treatments (Table 3, Factorial ANOVA, week effect), a significant interaction was found between time and conditions (Table 3, Factorial ANOVA, treatment x week effect). Significant differences in biomasses within treatments were present between week 3 and 6 of our nutrient and humic treatments (Figure 7B, $p < 0.05$, Tukey's Test).

Cyclopoid copepod species total abundance varied among treatments. *C. scutifer* showed no differences in total abundances within experimental treatments when compared to the control, however our nutrient treatment found higher total abundances when compared to our humic treatment (Fig. 8A, $p < 0.05$, Tukey's Test). *A. robustus* showed no significant differences in total abundance within or between treatments. There were no significant differences within treatments between week 3 and 6.

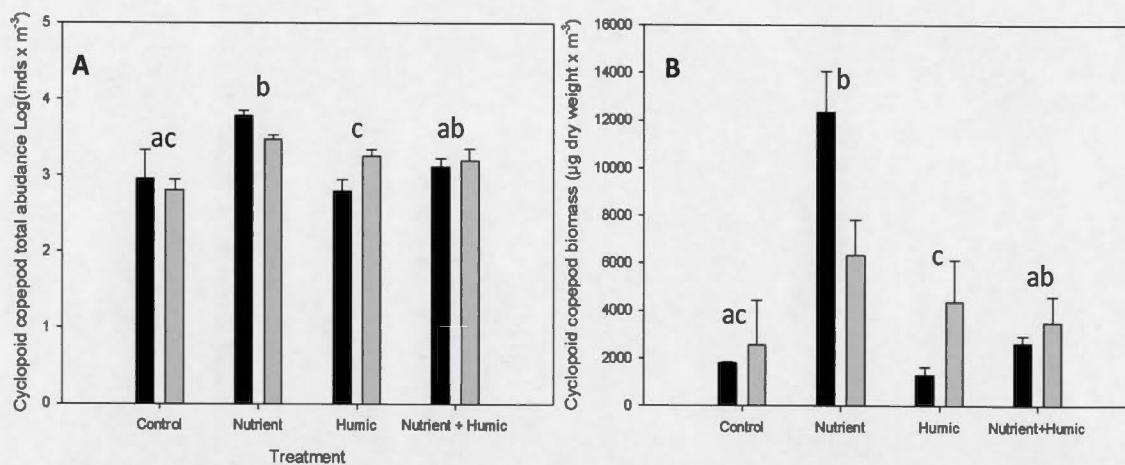


Figure 7. A) Cyclopoid copepod (*C. scutifer* and *A. robustus*) total abundance (mean Log(individuals per m³) \pm SE) and B) cyclopoid copepod (*C. scutifer* and *A. robustus*) biomass (mean μ g dry weight per m³) \pm SE) during week 3 (black) and week 6 (grey) of mesocosms. Results of post-hoc Tukey's test across treatments are indicated on figure by letters above bars.

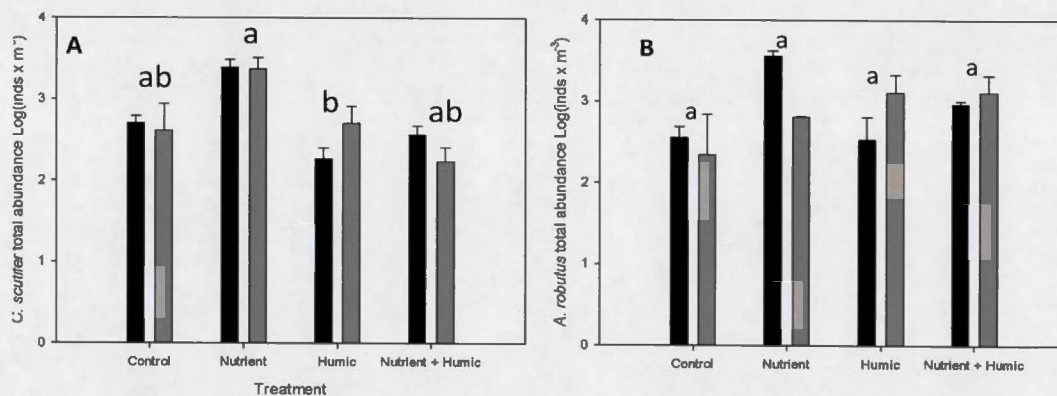


Figure 8. A) The cyclopoid copepod *C. scutifer* and B) *A. robustus* total abundance (mean Log(individuals per m³) \pm SE) during week 3 (black) and week 6 (grey) of mesocosms. Results of post-hoc Tukey's test across treatments are indicated on figure by letters above bars.

Table 3. Results of factorial ANOVA for crustacean zooplankton biomass among mesocosm treatments (n=4). Results of significance ($p < 0.05$) are bolded, and results of non-significance ($p > 0.05$) are denoted by ‘-’.

	Biomass			
	Crustacean Zooplankton	Calanoid copepods	Cladocerans	Cyclopoid copepods
Df effect	3	3	3	3
Df Error	32	32	32	32
Treatment				
F	50.729	31.106	42.103	17.865
P	<0.001	<0.001	<0.001	<0.001
Week				
F	6.141	--	6.277	7.878
P	<0.01	--	<0.005	<0.001
Treatment*Week				
F	4.526	--	4.589	4.423
P	<0.001	--	<0.001	<0.001

2.4.4 Crustacean zooplankton fatty acid response

Mesocosm water treatment appeared to elicit changes in the nutritional state of crustacean zooplankton. TAG Omega-3 (n-3) FA (18:3n-3, 18:4n-3c 20:3n-3, 20:4n-3, 20:5n-3, 22:6n-3, 22:3n-3, 22:5n-3) composition showed significant differences in calanoid copepods and cladocerans among treatments (Factorial ANOVA, Table 4, treatment effect). TAG Omega-6 (n-6) FA (20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6) composition did not show any differences among treatments. Ratios of n-3:n-6 were significantly different in calanoid copepod TAG composition among mesocosm water treatment (Factorial ANOVA, Table 4, treatment effect), however did not significantly vary for cladocerans. TAG Bacterial FA (15, 15:1n-5, 17, 17:1n-7, 14:Me13, 14:Me12, 15:Me14, 15:Me15, 16:Δ.9n-10, 18:1n-6, 18:Δ.9n-10) composition was significantly different among mesocosm water treatments for

calanoid copepods (Factorial ANOVA, Table 3, main effect), but significant differences were seen among water treatments for cladocerans. Replication was unequal among treatment and week (Table 5) due to insufficient animal tissue available at time of sampling.

Table 4. Results of factorial ANOVA for crustacean zooplankton FA profile composition (% TAG) among mesocosm treatments (n=4). Results of significance ($p < 0.05$) are bolded, and results of non-significance ($p > 0.05$) are denoted by '-'. '1' indicates a significant difference between treatments.

	Calanoid copepods				Cladocerans			
	n-3	n-6	n-3: n-6	Bacc FA	n-3	n-6	n-3: n-6	Bacc FA
Df effect	3	3	3	3	3	3	3	3
Df Error	8	8	8	8	11	11	11	11
Treatment								
F	69.75	--	7.289	5.09	4.160 1	--	--	--
P	<0.0001	--	<0.05	<0.05	<0.05	--	--	--
Week								
F	--	--	--	--	--	--	--	13.72
P	--	--	--	--	--	--	--	<0.01
Treatment* Week								
F	16.466	--	--	--	--	--	--	--
P	<0.01	--	--	--	--	--	--	--

Table 5. Replication of FA samples. Each sample is composed of >800 individuals from a mesocosm water treatment at an indicated point of sampling. Replication is equal to number of mesocosms with sufficient animal tissue to perform a laboratory analysis.

Replication present	Week 3		Week 6	
	Calanoid copepods	Cladocerans	Calanoid copepods	Cladocerans
Control	2	3	2	2
Nutrient	3	3	3	3
Humic	1	1	1	3
Nutrient + humic	3	3	1	1

TAG n-3 FA composition in crustacean zooplankton

Significant differences were found among mesocosm water treatments for calanoid copepod TAG n-3 FA composition (Fig. 9A). Calanoid copepods (*L.minutus*) of our nutrient water treatments had the highest retention of TAG n-3 FA composition over a 3 and 6 week time span (Tukey's Test, Figure 9A, $p < 0.0005$), followed by our nutrient + humic treatment (Tukey's Test, Figure 9A, $p < 0.05$). Significant differences within TAG n-3 FA composition between weeks 3 and 5 were only seen in our control treatment (Fig. 9A). Significant differences of TAG n-3 FA composition were found among mesocosm water treatments for cladocerans. Nutrient treatments showed a higher cladoceran TAG n-3 composition than our control and humic treatments (Tukey's Test, Fig. 9B, $p < 0.05$). No significant differences were found within weeks 3 and 6 of our water treatments for cladocerans (Tukey's Test, Fig. 9B).

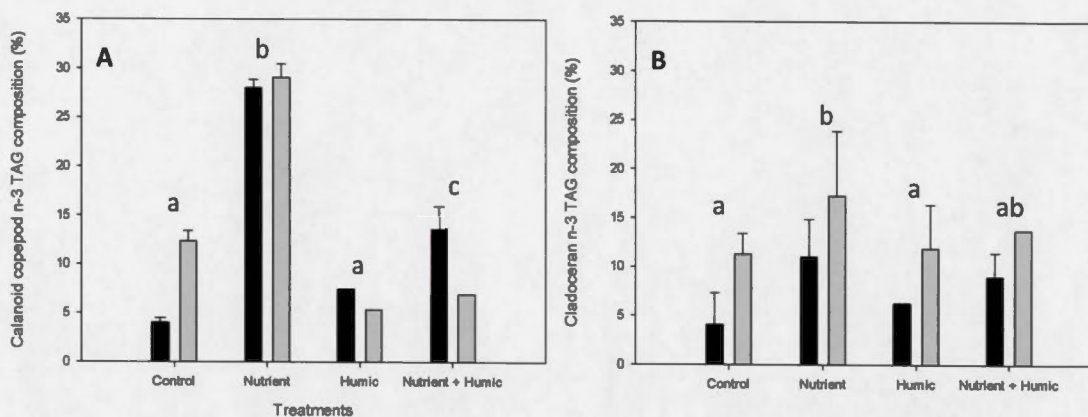


Figure 9. N-3 FA (mean \pm SE; percentage TAG composition) of A) calanoid copepods (*L. minutus*) and B) cladocerans (*D.ambigua*, *D.longiremis*, *S. lieder*) at week 3 (black) and 6 (grey) of the experiment. Results of post-hoc Tukey's test across treatments are indicated on figure by letters above bars.

TAG n-3:n-6 FA composition in crustacean zooplankton

Differences were found among mesocosm water treatments in calanoid copepod TAG n-3:n-6 ratios. Higher ratios were found in our nutrient treatments compared to our control and humic treatments (Tukey's Test, Figure 10A, $p < 0.05$). No differences were seen between our nutrient and nutrient + humic treatments. No significant differences in TAG n-3:n-6 ratios were found within treatments between weeks 3 and 6.

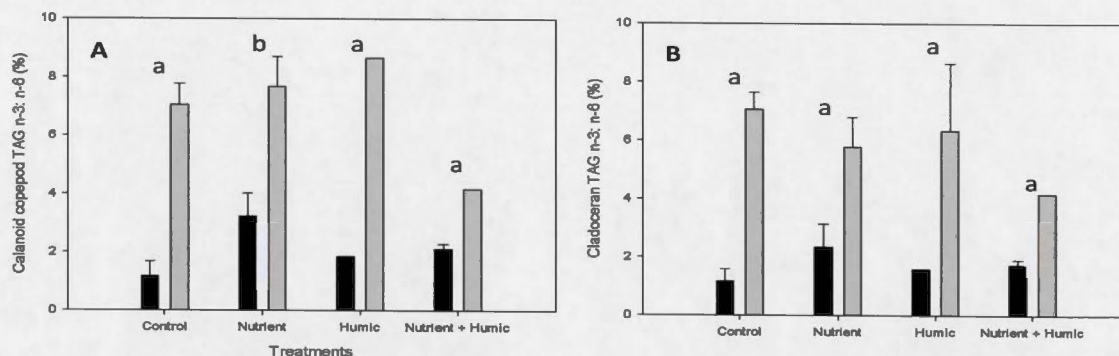


Figure 10. N-3:n-6 FA ratios (mean \pm SE; percentage TAG composition) of A) calanoid copepods (*L. minutus*) and B) cladocerans (*D. ambigua*, *D. longiremis*, *S. liederii*) at week 3 (black) and 6 (grey) of the experiment. Results of post-hoc Tukey's test across treatments are indicated on figure by letters above bars.

TAG bacterial FA composition in crustacean zooplankton

Differences were found among mesocosm water treatments in calanoid copepod TAG bacterial FA composition. Nutrient treatments had increased TAG bacterial FA composition when compared to all other treatments (Tukey's Test, Fig. 11A, $p < 0.05$). No differences were seen within treatments between weeks 3 and 6. No significant differences were seen among mesocosm water treatments for cladocerans. Differences were seen within treatments between weeks 3 and 6 for our nutrient treatment for cladocerans (Tukey's Test, 11B, $p < 0.05$).

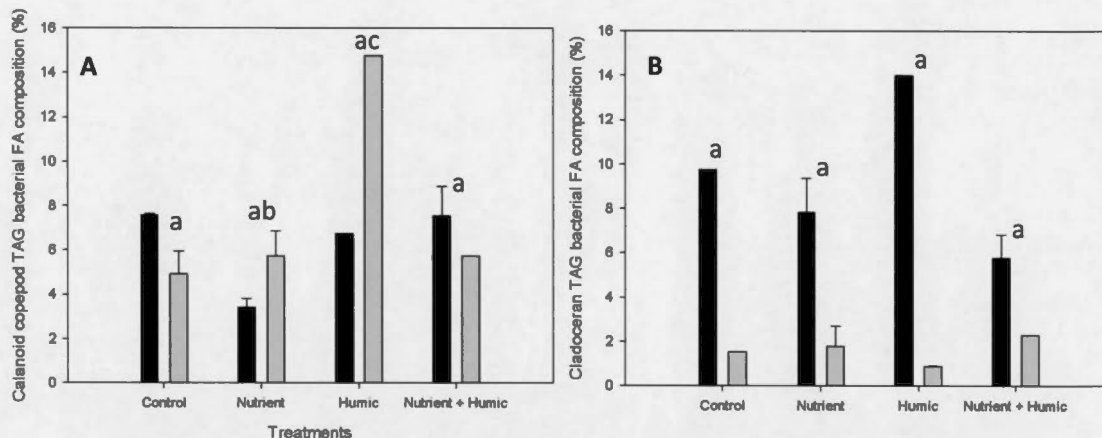


Figure 11. Bacterial FA (mean \pm SE; percentage TAG composition) of A) calanoid copepods (*L. minutus*) and B) cladocerans (*D. ambigua*, *D. longiremis*, *S. liederii*) at week 3 (black) and 6 (grey) of the experiment. Results of post-hoc Tukey's test across treatments are indicated on figure by letters above bars.

2.5 Discussion

Altered basal resources in divergent resource environments can alter community composition and nutritional state of crustacean zooplankton. The results of this study reflect the ecological consequences of stimulating or suppressing a green pathway rather than stimulating a brown food web pathway via humic addition. The first hypothesis, that nutrient addition will promote algal growth (green pathway), resulting in higher zooplankton abundance and higher concentrations of algal-limited essential fatty acids such as PUFAs was supported by my data. The second hypothesis, that humic addition would promote a brown food web pathway was not supported because while humic addition reduced zooplankton biomass, there was no evidence that basal resources were detrimentally affected in relation to the control

that had zooplankton with similar concentrations of essential fatty acids. Nutrient and humic addition produced an intermediate response as anticipated, because zooplankton biomass and TAG n-3 composition fatty acids were intermediate to treatments with only nutrient or humic addition. Bacterial fatty acids surprisingly occurred in similar concentrations in all zooplankton groups across water treatment. In some cases, the response of crustacean zooplankton to the water treatment was dependent on the taxonomic group, and likely different feeding strategies. Calanoid copepods showed the most pronounced differences in essential fatty acids among levels of water treatment compared to cladocerans, for which no differences in N-3:n-6 FA ratios were detected. This was an unanticipated result because selectively feeding calanoid copepods would be expected to show less differences among resource environments compared to passively feeding cladocerans. Differences in zooplankton nutritional state that are provoked by different resource environments can influence fecundity (Kainz *et al.*, 2004), and have possible ecological consequences for populations and communities.

2.5.1 Basal Resources: Phytoplankton and Bacteria

Nutrient addition had a positive effect on algal and bacterial production, and humic addition dampened the effects of nutrients on the green pathway. Nutrient addition created a highly productive, eutrophic environment with increased total phosphorus (TP), total nitrogen (TN), chlorophyll-*a*, gross primary production (PP_r), and bacterial production (BP) in reference to all other treatments. The addition of

nutrients reinforced a food web hypothesis known as the “green pathway” (Paul & Meyer, 2001) in which zooplankton diet is comprised of P-limited phytoplankton and algae. Most bacteria are also P-limited (Vrede *et al.*, 1999; Vadstein, 2000), which is consistent with the higher BP under conditions of nutrient addition. Humic addition resulted in shading, reduced light penetration, increased dissolved organic carbon (DOC), but no changes in the concentrations of TP or TN relative to the control treatment. Basal resource responses to humic additions included decreased gross PPr and respiration (Fig. 3), as well as reduced edible phytoplankton richness and density, but there was no change in chl-*a* or BP relative to the control. The nutrient + humic addition created an environment with reduced light penetration, increased DOC, and increased TP & TN in reference to the control. This treatment increased chlorophyll-*a* concentrations, gross PPr and BP relative to the control. It is known that PPr benefits from both N and P additions to avoid limitation (Elser *et al.*, 2007), and that bacteria are not only DOC limited, but P- limited as well (Hessen 1992; Vrede *et al.*, 1999; Vadstein, 2000). Experimental work on marine ecosystems also found evidence that DOC addition can dampen eutrophication from nutrients, in which DOC shifted algal communities to smaller species and increased bacterial biomass and heterotrophy (Andersson *et al.* 2013).

2.5.2 Crustacean Zooplankton

The response in abundance and biomass of different taxonomic groups of crustacean zooplankton to water treatment was likely affected by feeding (active

versus passive) and reproductive strategy. Calanoid copepods are selective feeders (Burns & Schllenburg, 2001) that can actively exclude low quality resources from their diet (Zelles, 1999). Calanoid copepod responses to water treatment likely reflect survival or mortality of adult and juvenile stages rather than fecundity since the reproductive cycle of copepods is approximately one month (Thorp and Covich 2010), the approximate duration of the experiment. Cladocerans are passive, filter feeders, and responses to water treatment likely reflect a combination of both survival and fecundity since cladocerans can reproduce clonally over the duration of several days (Thorp and Covich 2010).

Nutrient addition caused crustacean zooplankton (calanoid copepods, cladocerans, and cyclopoid copepods) to increase in abundance and biomass. These results were not unexpected because previous studies have shown zooplankton abundance and biomass is well correlated with environmental parameters that reflect eutrophication such as TP and chlorophyll-*a* (Patalas, 1972; Sarnelle, 1992). These responses can be attributed to greater food availability to females (Edmondson, 1965; Comita & Anderson, 1959), thus providing nutrients for future broods (Patalas, 1972). Phytoplankton such as diatoms and green algae which have been shown to increase in abundance and diversity with nutrient addition, are excellent sources of these essential n-3 PUFA (Ahlgren *et al.*, 1990; Brett *et al.*, 2009; Faithfull *et al.*, 2011a, c). Calanoid copepod and cladoceran augmentation of n-3 PUFAs in their TAG lipid profiles in nutrient treatments were likely a result of an abundance of high

quality algal resources. Availability, as well as quality of food plays a large role in the successful maturation of juvenile crustacean zooplankton and the resource allocation for gravid females (Arts *et al.*, 2009). As previously mentioned, n-3 PUFA are strongly linked with important life history traits such as fecundity and development (Arts *et al.*, 2009).

Nutrient addition not only boosted primary production of algal resources, but also bacterial production in the experimental enclosures. The higher bacterial production that was found in nutrient enriched enclosures could have allowed for some trophic upgrading (Broglia *et al.*, 2003; Vera *et al.*, 2001; Burkholder & Glasgow, 1997) by zooplankton via feeding on ciliates (Desvillettes & Bec, 2009). Wenzel *et al.* (2012) found that filter-feeding cladocerans benefit from a diet rich in both algae and heterotrophic bacteria. Faithful *et al.* (2012) have suggested that copepods, as selective feeders, may also benefit from trophic upgrade due to feeding on bacterivorous, PUFA rich flagellates. Passive feeders such as cladocerans, may be more susceptible to directly ingesting low quality food within their environment such as bacteria (Faithful *et al.*, 2011a). Bacteria contain very few, to no n-3 PUFA and have been considered low quality food for zooplankton (Zelles, 1999). However, bacterial fatty acids were similarly assimilated in both calanoid copepods and cladocerans in my experiment across the different water treatments. Interestingly, nutrient addition did not boost n3:n6 essential fatty acids in cladocerans as in calanoid copepods relative to other water treatments. Crustacean zooplankton

biomass and nutritional state in nutrient-enriched enclosures benefited from high quantity and quality of algal resources.

Humic addition reduced the abundance and biomass of calanoid copepods and cladocerans, but not cyclopoid copepods that are known to be adapted to low food availability (Elgmork, 1978). Dissolved humic substances can act as mild biochemical stressors for zooplankton that can interfere with chemical processes within the body and decrease organism fitness (Steinberg *et al.* 2006). Humic addition also created a strong shading effect, limiting PPr and potentially reducing zooplankton biomass because of reduced resources (Faithfull *et al.*, 2011c). However, algal biomass (chl_a) and bacterial production were neither suppressed nor boosted by the humic addition relative to the control. It is likely that humic addition suppressed high quality phytoplankton species without affecting total algal biomass (Alghren *et al.*, 1990). Reduced light penetration and high PPr shifted the phytoplankton community to be comprised of mostly inedible taxa (*Asteronella sp.*, *Synedra sp.*, *Tabellaria sp.*, *Rhizosolenia sp.*) (Appendix 1), that were not accessible as a resource for herbivorous zooplankton (calanoid copepods, cladocerans) (Burns & Schllenburg, 2001; Hambright *et al.*, 2007). However, in spite of these shifts in the algal community, I did not detect reduced n-3 and n-3:n-6 fatty acids in calanoid copepods and cladocerans in humic addition enclosures relative to control enclosures. For calanoid copepods, it is possible that ingestion of bacterivores (e.g. dinoflagellates) provided some trophic upgrading because dinoflagellates have the biocapacity to

synthesize PUFAs (Bec *et al.*, 2006; Desvillettes & Bec, 2009). Cladocerans may be able to use parthenogenetic reproductive strategies to compensate during conditions of environmental stress (Wacker & Martin-Creuzburg, 2007) as cladocerans often dominated across enclosures with humic addition. Cyclopoid copepods are omnivores (Wiujamson, 1984), and likely shifted their diet from phytoplankton to other zooplankton species, thus overcoming limited energy transfer (Faithfull *et al.*, 2011c). The humic addition therefore likely suppressed the green pathway and reduced the number of herbivorous zooplankton supported by this resource, but the nutritional state of individuals that remained was not compromised and did not depend on the feeding strategy of the zooplankton group.

Nutrient and humic addition caused responses in the biomass and nutritional state of crustacean zooplankton that were intermediate to the addition of nutrients and humic substances alone. Cyclopoid copepods emerged as the most successful order by measure of abundance and biomass in the nutrient + humic treatments, whereas calanoid copepods and cladocerans suffered; these responses were most evident by week 6. The dwindling zooplankton abundance (excluding cyclopoid copepods) and diversity that resulted from humic addition in spite of co-occurring nutrient addition appear to agree with Brett *et al.* (2009) whose work has shown, that it is phytoplankton, not allochthonous carbon, that sustains herbivorous zooplankton production. In enclosures with nutrients + humic addition treatment, calanoid copepods exhibited significant losses of PUFAs compared to control enclosures. As a

result of the reduced abundance of high quality phytoplankton and absence of dinoflagellates (Appendix 1), the effects of humic substances outweighed positive effects of nutrients on nutritional state of herbivorous crustacean zooplankton.

2.5.3 Implications

My experiment is among the first designed to understand effects of divergent resource environments on both crustacean zooplankton community structure and nutritional state (+ or – green pathway), whilst allowing for inter-specific competitive interactions that could influence resource acquisition and relative species abundance in whole zooplankton communities. The results of my work indicate that 1) crustacean zooplankton biomass and nutritional state are elevated in nutrient-enriched environments, and 2) humic addition reduces the quantity and quality of algal resources available to herbivorous zooplankton, reducing the diversity and biomass but not necessarily the nutritional state of individuals that remain.

The association between resource environment and zooplankton nutritional state that I found, as measured by essential fatty acids, reinforce the possibility for ecosystems to have different capacities for trophic capacity based upon basal resources (Mariash *et al.*, 2011). The novel limitations of essential PUFAs in zooplankton communities may have consequences for the transfer of these essential compounds to higher consumers such as predators, and overall the trophic capacity of an ecosystem (Kainz *et al.*, 2004). Follow-up research could measure patterns in

zooplankton fatty acids along landscape gradients in nutrients and dissolved organic carbon. TAG lipid profiles, the source for maternal investment lipids (Sperfeld & Wacker, 2012), could be followed up in future work by examining polar and TAG lipid profiles for a comprehensive understanding of energy allocation in low and high quality food environments.

CHAPITRE III

3.1 Concluding Chapter

Distribution of energy reserves in food webs and among organisms with different ecosystem functions could play important roles in species responses to climate and land use changes (Hoffmann & Sgro, 2011). Increasing global temperatures associated with climate change are predicted to increase input of terrestrially derived (allochthonous) DOC to freshwater lakes as vegetation in watersheds and precipitation increases in northern regions (Larsen *et al.*, 2011). With development and land use changes, increased phosphorus and nitrogen loading into freshwater systems will alter the base of the food web via eutrophication (Edmondson & Lehman, 1979; Tranvik *et al.*, 2009; Schindler *et al.*, 1977;). Increased DOC in freshwater lakes could also alter resource availability at the base of the food web via shading and bacterial productivity increases with potential cascading effects to higher trophic levels (Brett & Goldman, 1996; Kankaala *et al.*, 2010; Lennon *et al.*, 2013; Rautio & Vincent, 2006). With humic enrichment of aquatic ecosystems, bacterial

productivity is anticipated to increase and could potentially be incorporated as a substantial portion of the grazing zooplankton diet (Bergstrom & Jansson, 2000; Kankaala *et al.*, 2010), with possible detrimental consequences for population-level fecundity and abundance, and community interactions (Faithfull *et al.*, 2011a).

In my study, manipulation of nutrients and humic substances altered algal and bacterial basal resources, but not always in the ways that were expected based on the literature. Although humic addition did increase DOC, it did not result in an increase in BP in the absence of nutrients, but did reduce algal biomass and primary production through shading. As expected, when nutrients (phosphorus and nitrogen) were added, both BP and phytoplankton biomass and production increased. When immersed in a eutrophic environment without humic addition, calanoid copepods retained high amounts of n-3 and n-6 PUFAs, as well as increased total abundance. Cladocerans showed similar trends as calanoid copepods in the nutrient addition treatment and the humic addition treatment. Calanoid copepods and cladocerans of the nutrient + humic treatment showed no significant loss of n-3 PUFAs when compared to the reference control treatment while experiencing a decreased in total abundance. Eutrophic conditions appeared to increase crustacean zooplankton abundance and improve nutritional state via supporting n-3 rich basal resources. Essential lipids such as n-3 PUFAs were tightly linked with environments rich with phytoplankton and photosynthetic activity.

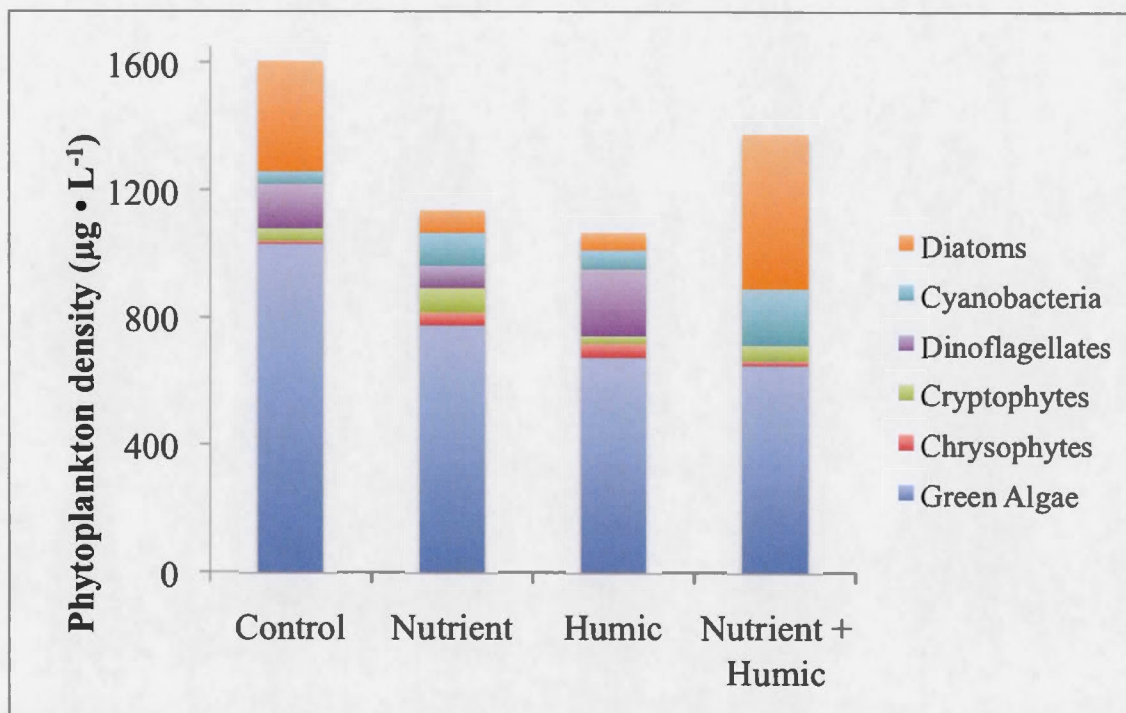
With increased amount of n-3s, an organism can experience better survivorship, and next generation success (Arts *et al.*, 2009). In crustacean zooplankton, there is strong evidence for reproductive dependency on essential n-3 PUFA. Lochmann *et al.* 2007, demonstrates that when crustacean zooplankton increase essential n-3s PUFAs, such as EPA and DHA, fecundity and other key life history traits such as are improved. With anticipated changes in the physico-chemical conditions of fresh waters in the future (Jeppesen *et al.*, 2011), and by association basal resources (Faithfull *et al.*, 2001b), crustacean zooplankton community composition, abundance, and nutritional state may also change. While eutrophied environments will likely support high numbers of healthy crustacean zooplankton, humic-enriched ecosystems may experience decreased zooplankton abundance and reduced richness, limiting EFA in freshwater ecosystems.

The novel limitations of essential PUFAs in zooplankton communities may have consequences for the transfer of these essential compounds to higher consumers such as predators, and overall the trophic capacity of an ecosystem (Kainz *et al.*, 2004; Arts *et al.* 2009). Reduced zooplankton biomass and PUFA-limitation may be especially extreme in humic ecosystems with low nutrients, where there can potentially be suppression of overall ecosystem production Karlsson *et al.* (2009). Moreover, PUFA limitations in food webs can result from inter-specific competition between crustacean zooplankton, in which the success or failure of a specific species may change essential PUFA availability to predators such as juvenile fish (Gladyshev

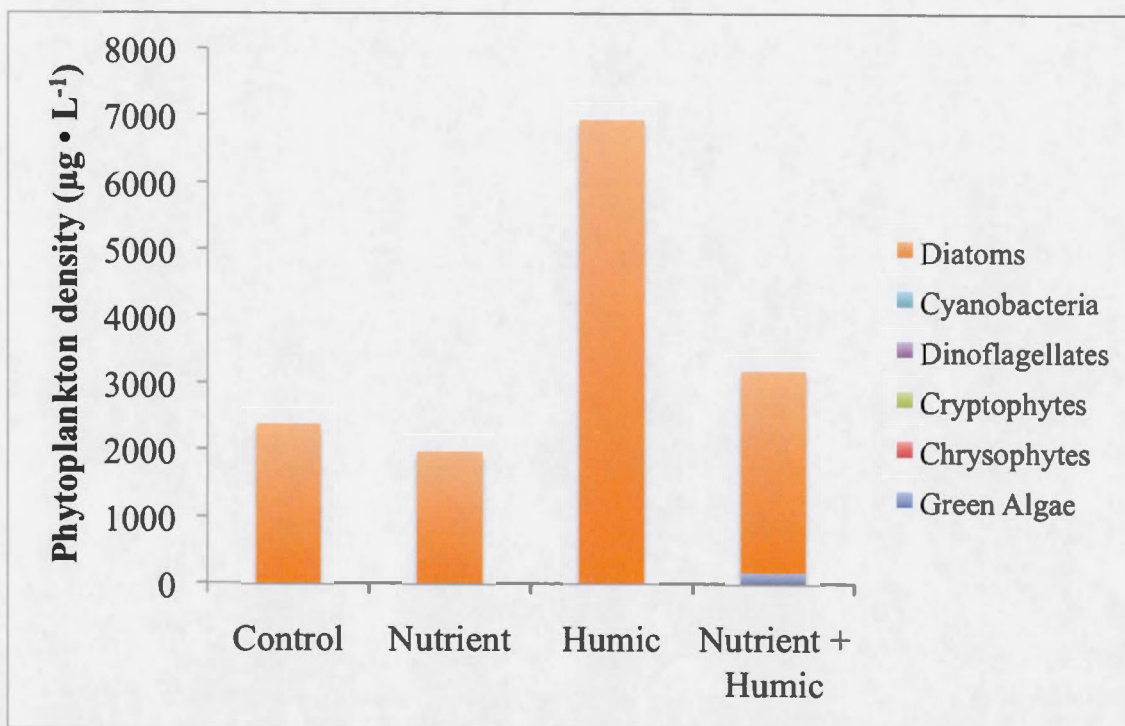
et al., 2009; Lau *et al.*, 2012; Lochmann *et al.*, 2007). This is because the PUFA content of crustacean zooplankton varies between major groups such as calanoid copepods, cladocerans, and cyclopoid copepods (Sekino *et al.*, 1997). The assessment of nutritional states of crustacean zooplankton and their composition of aquatic communities can therefore provide a comprehensive understanding of ecosystem health (Arts *et al.*, 2009; Kainz *et al.*, 2004).

Despite the potential for cascading effects in aquatic food webs, no other study has investigated the effects of contrasting resource pathways associated with nutrient and humic addition on the nutritional state of crustacean zooplankton, especially in a natural non-laboratory setting. Because essential FAs such as n-3 PUFAs can be limited or enhanced by indirect manipulation of basal resources through the addition of nutrients or DOC, we gain insight on how land use and climate change could affect community composition, abundance, and nutritional state of crustacean zooplankton communities. If these changes in crustacean zooplankton occur in real ecosystems in nature in association with these environmental variables, there is high potential for cascading effects related to energy transfer of essential PUFAs at higher trophic levels in aquatic food webs. My study uniquely highlights the importance of considering ecological responses at both community and biochemical levels when assessing biological consequences of environmental change.

ANNEXE I



Summary of edible (<35 µm) phytoplankton assemblages across the 3-week mesocosm treatments. Each phytoplankton order is represented in density (µg L⁻¹). Density was based upon integrated sampling technique and volume sampled.



Summary of inedible (>35 µm) phytoplankton assemblages across the 3-week mesocosm treatments. Each phytoplankton order is represented in density (µg L⁻¹). Density was based upon integrated sampling technique and volume sampled.

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